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ABSTRACT

The instructor's guide, the first of three documents in this package, is for a course to help students who are investigating the activities within a hospital, clinic, or physician's office. The material is designed to relate training experience to information studied in the classroom. The course is intended for individualized study and is competency based with educational outcomes stated for each unit. The instructor's manual provides a list of essential elements correlated to the units of instruction. A basic test, answers to tests, and answers to learning activities are included for each unit, and a competency chart is included. The materials are organized in 11 units: safety in the laboratory; organization of the laboratory; collecting and processing specimens; laboratory equipment; urinalysis; hematology; serology; microbiology; chemistry; the blood banks; and histology and cytology. The student's manual, the second of the three documents, is designed for use by students investigating the activities within a medical laboratory in a hospital, clinic, or physician's office. The material is designed to relate training experience to information studied in the classroom. It is designed for individualized study and is competency based, with educational outcomes stated for each unit. The student's manual contains learning objectives terms and definitions, and information relating to current practices. Topics addressed are: the organization, personnel, and types of equipment used in laboratories. Collection and processing of specimens are described. Techniques, both manual and automated, are discussed for the following departments: urinalysis, hematology, serology, microbiology, chemistry, the blood bank, and histology and cytology. Safety and quality control are emphasized. The illustrated units consist of objectives, terms and definitions, information sheets, and a summary. The manual also contains a list of 53 medical laboratory abbreviations and a bibliography listing 18 references. Individualized student learning activities are provided (in the last of the three documents) to encourage students to read and study the information presented in the medical laboratory assisting course. One or more learning activities are included for each of the 11 course units. Materials consist of questions to answer, suggested research activities, matching tests, and labeling activities. (KC)

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ASSISTING IN THE MEDICAL LABORATORY

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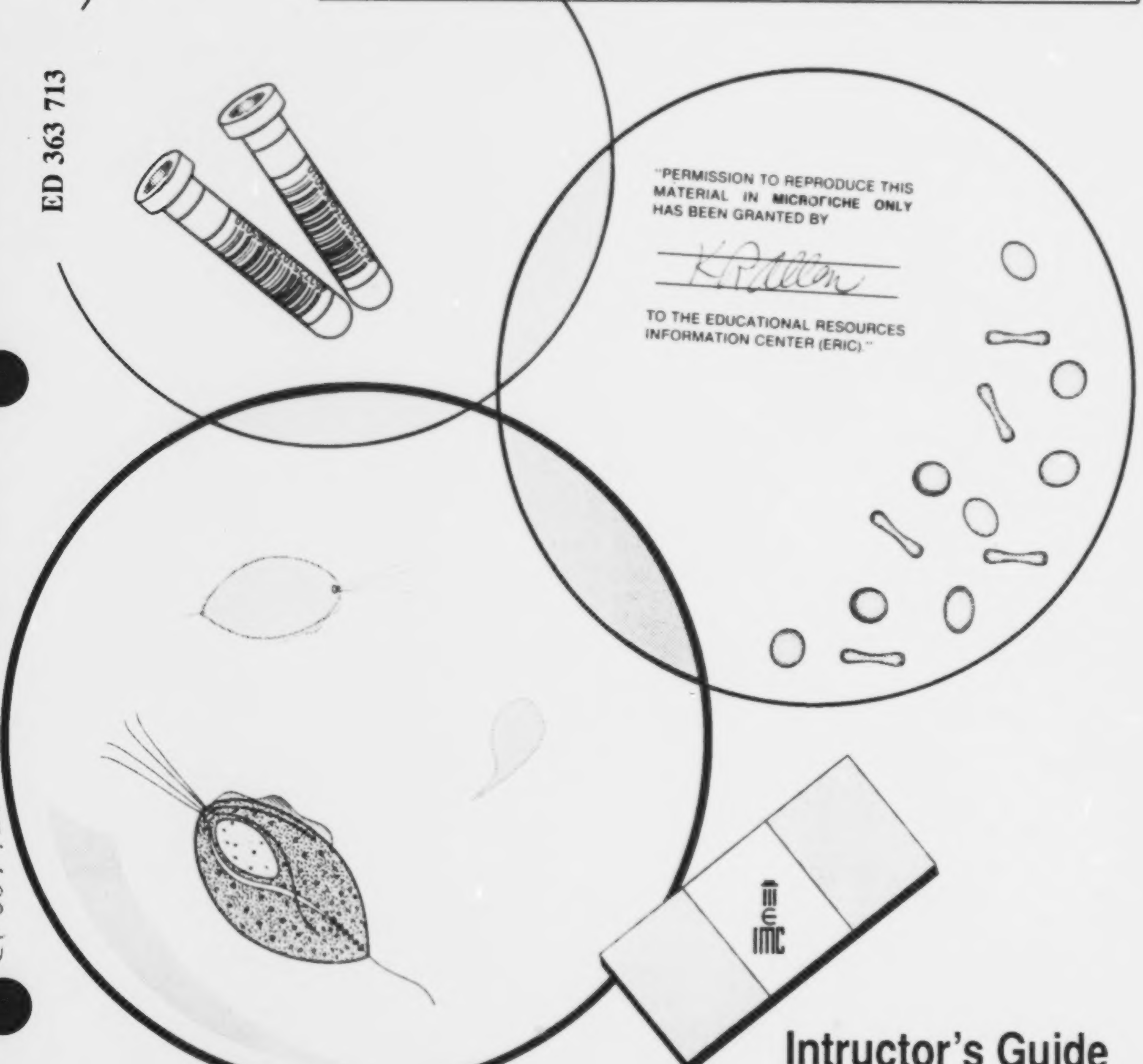

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Intructor's Guide

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Assisting in the Medical Laboratory

Instructor's Guide

By

Helena J. Fair

Published by

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COMPLIANCE STATEMENT

TITLE VI, CIVIL RIGHTS ACT OF 1964; THE MODIFIED COURT ORDER, CIVIL ACTION 5281, FEDERAL DISTRICT COURT, EASTERN DISTRICT OF TEXAS, TYLER DIVISION

Reviews of local education agencies pertaining to compliance with Title VI Civil Rights Act of 1964 and with specific requirements of the Modified Court Order, Civil Action No. 5281, Federal District Court, Eastern District of Texas, Tyler Division are conducted periodically by staff representatives of the Texas Education Agency. These reviews cover at least the following policies and practices:

(1) acceptance policies on student transfers from other school districts;

(2) operation of school bus routes or runs on a non-segregated basis;

(3) nondiscrimination in extracurricular activities and the use of school facilities;

(4) nondiscriminatory practices in the hiring, assigning, promoting, paying, demoting, reassigning, or dismissing of faculty and staff members who work with children;

(5) enrollment and assignment of students without discrimination on the basis of race, color, or national origin;

(6) nondiscriminatory practices relating to the use of a student's first language; and

(7) evidence of published procedures for hearing complaints and grievances.

In addition to conducting reviews, the Texas Education Agency staff representatives check complaints of discrimination made by a citizen or citizens residing in a school district where it is alleged discriminatory practices have occurred or are occurring.

Where a violation of Title VI of the Civil Rights Act is found, the findings are reported to the Office for Civil Rights, Department of Health, Education and Welfare.

If there is a direct violation of the Court Order in Civil Action No. 5281 that cannot be cleared through negotiation, the sanctions required by the Court Order are applied.

TITLE VII, CIVIL RIGHTS ACT OF 1964: EXECUTIVE ORDERS 11246 and 11375; TITLE IX 1973 EDUCATION AMENDMENTS: REHABILITATION ACT OF 1973 AS AMENDED; 1974 AMENDMENTS TO THE WAGE-HOUR LAW EXPANDING THE AGE DISCRIMINATION IN EMPLOYMENT ACT OF 1967; VIETNAM ERA VETERANS READJUSTMENT ASSISTANCE ACT OF 1972 AS AMENDED IN 1974.

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Notes for the Instructor

Assisting in the Medical Laboratory is designed for use by students investigating the activities within the medical laboratory in a hospital, clinic, or physician's office. The material is designed to relate training experience to information studied in the classroom. Information is provided which is current and relevant. The material is designed for individualized study and is competency-based with educational outcomes stated for each unit. *Assisting in the Medical Laboratory* is made up of three separate publications.

- The **Student's Manual** contains learning objectives, terms and definitions, and information relating to current practices. Topics addressed are the organization, personnel, and types of equipment utilized in laboratories. Collection and processing of specimens is described. Techniques, both manual and automated, are discussed for the following departments: urinalysis, hematology, serology, microbiology, chemistry, the blood bank, and histology/cytology. Safety and quality control are emphasized.

- The **Student Learning Activities** are individualized and designed to encourage the student to carefully read and study the information presented. Each unit has a section for those students desiring more advanced study. Instructors are encouraged to provide one set of activities for each student to facilitate learning.

- The **Instructor's Guide** has a list of essential elements addressed by the units of instruction. A basic test is provided for each unit. Instructors are encouraged to enlarge these. Answers to tests and learning activities are also provided, and a competency chart is included.

Acknowledgments

The Extension Instruction and Materials Center, Educational Resources, wishes to acknowledge with grateful appreciation the cooperation and assistance of the following health occupations teachers and professionals who served as members of the 1991-92 Health Occupations Project Advisory Committee:

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Portions of this book are adapted from two EIMC publications: *Medical Laboratory Assistant* (1981), by Sara Barnett, and *Medical Laboratory Services* (1981), by Catherine Williams.

Essential Elements Addressed by Units in *Assisting in the Medical Laboratory*

Below is a list of the units in *Assisting in the Medical Laboratory* that address the essential elements as specified in subsection 75.84 (p) and approved by the Texas State Board of Education, January, 1988. The essential elements are listed on the left. The name of the unit which presents that concept is listed on the right.

(k) Medical laboratory aide (2-3 units) shall include the health occupations core and the concepts and skills associated with the occupation of medical laboratory aide. The student shall be provided opportunities to:

(1) wash, rinse, and dry glassware and instruments using acceptable methods	Unit 1: Safety in the Laboratory Unit 4: Laboratory Equipment
(2) fill tubes and bottles with specified solutions, and apply identification labels	Unit 3: Collecting and Processing Specimens Unit 4: Laboratory Equipment Unit 9: Chemistry
(3) label and file microscope slides	Unit 6: Hematology Unit 11: Histology/Cytology
(4) arrange specimens and samples on trays to be placed in incubators and refrigerators	Unit 3: Collecting and Processing Specimens Unit 8: Microbiology Unit 10: The Blood Bank
(5) perform routine tests in medical medical laboratory under supervision of medical technologist or other qualified individual utilizing prescribed procedures	Unit 2: Organization of the Laboratory Unit 5: Urinalysis Unit 6: Hematology Unit 7: Serology Unit 8: Microbiology Unit 9: Chemistry Unit 10: The Blood Bank Unit 11: Histology/Cytology

Answers to Learning Activities

UNIT 1 / SAFETY IN THE LABORATORY

Learning Activity 1: Terms and Definitions

1. flammable
2. incident report
3. codes
4. Centers for Disease Control
5. CDC
6. hazard
7. corrosive
8. incinerated
9. safety
10. extinguish
11. legible
12. National Fire Protection Association
13. NFPA
14. OSHA
15. Occupational Safety and Health Act

Learning Activity 2: Safety Practices

1. OSHA, CDC
2. Secretary of Labor
3. to indicate an emergency situation and to keep from frightening patients and visitors
4. a. Code Yellow b. Code Green c. Code Blue d. Code Red
5. safety shower, eye wash station, hoods
6. Answers will vary with the facility.
7. wearing a lab jacket or coat and gloves
8. mask, goggles, face shield
9. a. supervisor
   b. description of accident, time and place, cause, names of witnesses
10. check the patient's identification
11. Bottles must be labeled. Labels must be legible. Always read bottle three times—as you reach for the bottle, as you pour, and as you replace it.

Learning Activity 3: Fire Safety

Answers 1 through 5 will vary with the facility.

6. a. to put out flash fires of clothing
   b. grasp rope handle, place right arm through rope loop, pull vigorously while turning to left
7. Code Red
8. Answers may vary with facility, but usually include supervisor and telephone operator.
9. a. C
   b. carbon dioxide, multipurpose dry chemical, ordinary dry chemical
10. a. multipurpose
    b. on all types of fires
11. a. green triangle; ordinary combustibles
    b. red square; flammable liquids, grease, oil
    c. blue circle; electrical equipment
12. a. Do not use water.
    b. foam, carbon dioxide, multipurpose dry chemical, ordinary dry chemical

Learning Activity 4: Contaminated Materials

1. Centers for Disease Control

2. blood, sputum, semen, vaginal secretions, urine, stool, tissues, drainage from open wounds
3. blood, cerebrospinal fluid, tears, etc.
4. Disinfect with bleach and autoclave. Place in a biohazard bag and incinerate.
5. Cover entire area with paper towels. Flood with bleach. Wearing gloves, place soaked towels and spillage on a container for autoclaving.
6. Place in biohazard bag and incinerate.
7. placed in puncture proof container in area where used; container is disposed of by local policy
8. Clean and disinfect your work area, remove gloves, remove lab coat or jacket, and wash your hands.
9. any worker exposed to infectious materials
10. employer
11. The outside is considered "clean" when donning gloves. So the gloved hand should touch only the clean side (outside) of the other glove to assist in fitting it over the hand.
12. symbol in center

Learning Activity 5: Precautions for Health Care Workers

1. persons, including students and trainees, whose activities involve contact with patients or with blood or other body fluids from patients in a health care setting
2. a well-constructed container with a secure lid
3. exposing bacteria to high-frequency sound waves
4. never
5. situations in which there is no alternative
6. first decontaminated or placed in bags and then disposed of in accordance with institutional policy
7. equipment decontaminated and cleaned
8. wash hands, remove all protective clothing before leaving the laboratory

UNIT 2 / ORGANIZATION OF THE LABORATORY

Learning Activity 1: Personnel of a Laboratory

Instructor must evaluate chart for accuracy and completeness.

Learning Activity 2: Levels of Education

1. g
2. d
3. f
4. b
5. a
6. c
7. e

Learning Activity 3: Laboratory Personnel

1. pathologist
2. internist
3. laboratory aide
4. phlebotomist
5. medical laboratory technician
6. cytologist
7. histologic technician
8. medical technologist
9. histotechnologist
10. pathologist
11. medical laboratory technologist

Learning Activity 4: Work Areas within the Laboratory

1. b
2. je
3. c
4. h
5. g
6. j
7. i or j
8. d
9. d
10. f
11. a or h
12.
13. f
14. a
15. f
16. b
17. b
18. i
19. a
20. d

Learning Activity 5: Computers in the Laboratory

1. electronic devices with ability to create, process, and store data
2. work station for a computer consisting of a video screen and keyboard
3. printed material
4. patient care, business management
5. Computers are placed at each nursing station and in each department. Orders can be put in the computer and sent to the laboratory. Reports can be sent out from the lab. Billing can be sent to the business office, and data to the record room.
6. preparing labels, running automatic equipment for testing, evaluating control procedures, reporting test results

Answers for questions 7 through 9 will vary with the facility.

UNIT 3 / COLLECTING AND PROCESSING SPECIMENS

Learning Activity 1: Terms Relating to the Processing of Specimens

1. apprehensive
2. hostile
3. rapport
4. biopsy
5. catheter
6. lancet
7. venipuncture
8. bevel
9. hemolysis
10. supernatant
11. plasma
12. substance to prevent blood from clotting
13. coagulation
14. serum
15. lipemic

Learning Activity 2: Interacting with Patients

"Things to Do" must include use name, listen, be friendly, explain procedure, allow patient to assist, remain calm, explain delays, immobilize small children and proceed rapidly, instruct patients for dismissal, and guard the privacy of patient records. More may be included.

"Things to Avoid" should include laughing at patient, arguing if patient refuses test, letting a patient wait without an explanation of delay, telling a child "this isn't going to hurt," not giving directions at dismissal, talking about patient records, and giving results of tests to patients. More may be included.

Learning Activity 3: Collection of Specimens

1. venipuncture
2. name tag, arm band, bed tag, ask patient's name
3. veins in the inner bend of the elbow
4. to prevent hemolysis
5. multiple samples are needed

6. oxalate, EDTA, sodium citrate, heparin
7. Tubes are color coded to indicate what is in tube.
8. (Any three of the following)
   Use sharp needles of appropriate size.
   Enter the vein directly.
   Do not tie tourniquet too tightly, and release it before aspirating blood.
   Remove needle before distributing blood into tubes.
   Allow blood to clot before centrifuging
9. earlobe or toe for adults, toe or heel for infants
10. Specimen cannot be contaminated with urine.
    No laxative unless ordered by the doctor.
    Provide wide-mouthed container with lid and collecting spatula or tongue blades.
    Patient may defecate in bedpan, disposable paper collection unit, or on a paper plate.
    Collect feces (size of pecan) into container, and cover.
    Patient must wash hands and label container.
11. gross examination for consistency, color, mucus, or blood; occult blood; detection of parasites and ova; viral and bacterial cultures
12. series of urine tests selected by a given institution to be performed on most urine specimens
13. first morning urine specimen, timed urine specimen
14. to diagnose disease, to determine treatment, to define progress of treatment
15. excretion, needle aspiration, resection, biopsy, smear
16. recording the specimen
17. color coding, numbers, adhesive labels, bar codes, request form, marking pencils
18. preparation of specimen for analysis
19. Examine specimen as quickly as possible after collection.
20. Few labs are equipped to conduct every test.
21. Answers will vary with the facility but should be definite and complete.

Learning Activity 4: Collection of Blood Samples

Venipuncture Using a Syringe and Needle	Collecting Capillary Blood from a Finger
A. 6	A. 5
B. 2	B. 3
C. 3	C. 2
D. 5	D. 1
E. 4	E. 4
F. 1	

Precautions for yourself: wear gloves and lab coat or jacket, provide equipment
Precautions for the patient: identify properly

UNIT 4 / LABORATORY EQUIPMENT

Learning Activity 1: Terminology Relating to Equipment

1. pipette
2. sterilize
3. autoclave
8. centrifuge
9. serological
10. graduated cylinder

4. thermostat
5. calibration mark
6. volumetric
7. meniscus
11. revolutions per minute
12. to contain
13. to deliver

Learning Activity 2: Identification of Equipment

1. aspirator bulb
2. beaker
3. volumetric flask
4. Florence flask
5. graduated cylinder
6. transfer pipette
7. volumetric pipette
8. serological pipette
9. Erlenmeyer flask
10. multiple-dose dispenser
11. dispenser
12. funnel

Learning Activity 3: Storage and Use of Equipment

1. efficiency in use, avoiding breakage
2. Reading of liquid volumes are made at the bottom of the meniscus. The meniscus is read against the calibration.
3. volumetric
4. a straight-sided container with a pouring spout formed by the rim; no
5. measure volumes of liquids, e.g., serological, volumetric
   transfer liquids from one container to another, e.g., volumetric , transfer
6. Attach a bulb to the mouthpiece, place the pipette in the solution, and use suction to draw the solution up until the meniscus is on the mark. Wipe outside of pipette to dry it. Place tip of pipette against side of receiving vessel. Release suction and allow liquid to drain.
7. Centrifuges spin at high speeds. Centrifugal force causes substances of different densities to separate, with heavier particles falling to the bottom, leaving fluids and lighter particles above.
8. to separate serum and plasma from whole blood, to separate bacteria from liquid media, and to concentrate urine sediment for microscopic study
9. microhematocrit centrifuge, refrigerated centrifuge, and tabletop centrifuge with variable speed or time
10. for constant results to meet quality control standards
11. tachometer, stroboscope, vibrating reed tachometer
12. hot-air oven—for glassware or metal instruments
    autoclave—for glassware, sharp-edged or hinged instruments, water, culture media, or linens
13. water bath, heating block

Learning Activity 4: Use of the Microscope

1. carrying arm
2. objectives
3. slide clip
4. condenser adjustment wheel
5. fine adjustment wheel
6. coarse adjustment wheel
7. microscope base
8. substage light
9. switch
10. iris diaphragm lever
11. diaphragm unit
12. stage
13. nosepiece
14. barrel
15. ocular

16. A monocular microscope has one eyepiece, while a binocular has two.
17. electron microscope
18. The coarse adjustment wheel moves the barrel or stage much more than the fine adjustment wheel does.
19. to avoid breaking slides, cover slips, or lenses
20. mirror, substage lamp
21. that the magnification increases the size of an object 10 times
22. 200X
23. (a) lens paper, (b) xylene/xylol on lens paper
24. Place one hand on the carrying arm and the other under the base.

UNIT 5 / URINALYSIS

Learning Activity 1: Terms Relating to Urinalysis

1. a. anuria—absence of excretion of urine from body
   b. hematuria—presence of blood in urine
   c. polyuria—excessive urination
   d. pyuria—excessive number of white cells (pus) in urine
2. *Macroscopic* means that something is visible to the human eye. *Microscopic* means that something is visible if using a microscope.
3. a. void b. urinate
4. a. substance that separates out of solution and settles to the bottom
   b. decant—pour off liquid so that sediment remains in bottom of container
   c. (1) amorphous—without a definite shape
      (2) artifact—an artificial structure
      (3) casts—cylindrical structures
      (4) occult blood—minute amount of blood
5. rolled together or coiled
6. water
7. A quantitative test determines how much of a substance is present. A qualitative test indicates that a substance is present.

Learning Activity 2: Scrambled Terms

1. specific gravity
2. quantitative
3. void
4. sediment
5. casts
6. supernatant
7. occult
8. microscopic
9. decant
10. urinalysis

Learning Activity 3: Collecting Specimens and Performing Macroscopic Urinalysis

1. a. nursing service b. laboratory personnel
2. first morning specimen, random specimen
3. Bacteria multiply rapidly, and leukocytes and casts tend to disintegrate at room temperature.
4. patient's name, doctor, date, test requested
5. room number, chart number or hospital number
6. Pleasantly instruct the patient to collect another specimen in the clinic rest room facility, and provide a clean container.

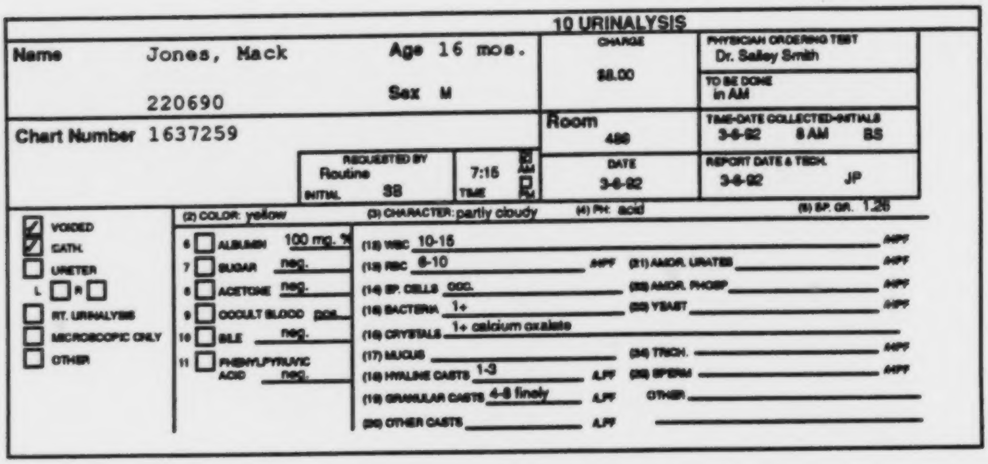
7. color, character
8. a. dyes, medications
   b. very dilute
   c. bilirubin, iron, porphyrin, melanin, or medications
   d. concentrated, medications
9. appearance or transparency
10. clear, partly cloudy, cloudy, grossly bloody
11. crystals, white blood cells, blood
12. 1.010 to 1.020
13. large quantities of urine produced
14. refractometer, urinometer
15. refractometer
16. acidity or alkalinity of urine, 5 to 9
17. use of commercial "stix"
18. use of commercial "stix"
19. protein, glucose, ketones, blood, bilirubin, urobilinogen, nitrites, phenylketones
20. Dip stix in fresh urine. Tap stix against the container side. Observe the specified times and read accordingly. Compare to color chart. Record results.

Learning Activity 4: Microscopic Urinalysis

1. Mix the urine by swirling gently to redistribute any particles that may have settled out. Pour 10 to 12 ml of well-mixed urine into a centrifuge tube, and centrifuge at 1,500 to 2,000 rpm for 5 minutes. Pour the liquid out of the tube; then place the tube in an upright position so that the remaining fluid on the sides flows to the bottom to dilute the sediment. Mix the remaining fluid and the sediment by gently tapping the tip of the tube on your hand. Pour a small amount on a clean slide, and cover with a cover slip.
2. to make cellular structures easier to see
3. careless transfer of sediment, use of too much light in the microscopic examination, use of high power only, drying of sediment, dirty equipment, scratches on the slide
4. (a) low-power field and high-power field; (b) by an average of what is seen in 10 fields;
   (c) RBC, WBC, sperm, Trichomonas, casts (any three)
5. Answers will vary with the institution.
6. epithelial cell, RBC, WBC, bacteria, spermatozoa, Trichomonas, yeast
   Three of these should have sketches. Refer to the Student's Manual.
7. Trichomonas is a parasite from vaginal secretions found in urinary sediment. It is not normal.
8. For sketches, see the Student's Manual.
   a. hyaline cast—cylindrical and transparent
   b. finely granular cast—contains fine granules
   c. coarsely granular cast—contains coarse granules
   d. red cell cast—composed of RBCs
   e. white cell cast—composed of WBCs
   f. fatty cast—contains fat droplets
   g. waxy cast—opaque, and may be convoluted
9. cylindroids, mucous threads. Also, nylon, cotton, or vegetable fibers may be mistaken for casts or mucous threads.

10. uric acid, calcium oxalate, amorphous urates, calcium carbonate, tyrosine needles, leucine spheres, sulfanilamide medication forms
11. amorphous phosphates, triple phosphate, calcium phosphate, calcium carbonate, ammonium biurate
12. talc, starch granules

Learning Activity 5: Reporting Urinalysis



10 URINALYSIS

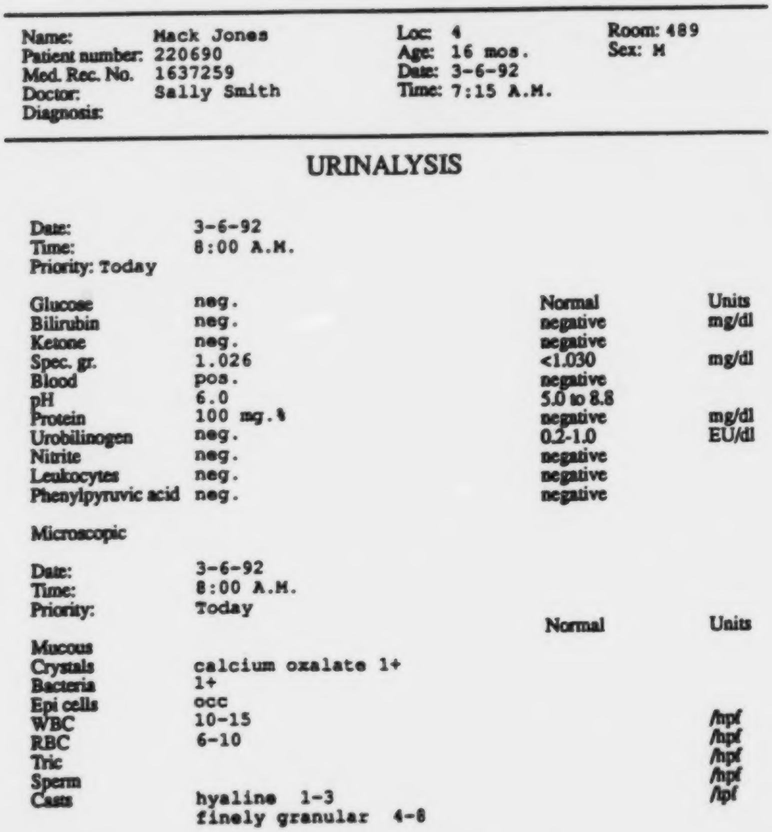
Name Jones, Mack Age 16 mos.
220690 Sex M
Chart Number 1637259
REQUESTED BY Routine
INITIAL SB
TIME 7:15 ☒ AM ☐ PM
CHARGE $8.00
Room 489
DATE 3-6-92
PHYSICIAN ORDERING TEST Dr. Salley Smith
TO BE DONE in AM
TIME-DATE COLLECTED-INITIALS 3-6-92 8 AM BS
REPORT DATE & TECH. 3-6-92 JP

☑ VOIDED
☑ CATH.
☐ URETER
L ☐ R ☐
☐ RT. URINALYSIS
☐ MICROSCOPIC ONLY
☐ OTHER

(2) COLOR: yellow
(3) CHARACTER: partly cloudy
(4) PH: acid
(5) SP. GR. 1.26

6 ☐ ALBUMIN 100 mg. %
7 ☐ SUGAR neg.
8 ☐ ACETONE neg.
9 ☐ OCCULT BLOOD pos.
10 ☐ BILE neg.
11 ☐ PHENYLPYRUVIC ACID neg.

(12) WBC 10-15 /HPF
(13) RBC 6-10 /HPF
(14) EP. CELLS occ.
(15) BACTERIA 1+
(16) CRYSTALS 1+ calcium oxalate
(17) MUCUS
(18) HYALINE CASTS 1-3 /LPF
(19) GRANULAR CASTS 4-8 finely /LPF
(20) OTHER CASTS /LPF
(21) AMOR. URATES /HPF
(22) AMOR. PHOSP /HPF
(23) YEAST /HPF
(24) TRICH. /HPF
(25) SPERM /HPF
OTHER



Name: Mack Jones
Patient number: 220690
Med. Rec. No. 1637259
Doctor: Sally Smith
Diagnosis:

Loc: 4
Age: 16 mos.
Date: 3-6-92
Time: 7:15 A.M.

Room: 489
Sex: M

URINALYSIS

Date: 3-6-92
Time: 8:00 A.M.
Priority: Today

		Normal	Units
Glucose	neg.	negative	mg/dl
Bilirubin	neg.	negative	
Ketone	neg.	negative	
Spec. gr.	1.026	<1.030	mg/dl
Blood	pos.	negative	
pH	6.0	5.0 to 8.8	
Protein	100 mg.%	negative	mg/dl
Urobilinogen	neg.	0.2-1.0	EU/dl
Nitrite	neg.	negative	
Leukocytes	neg.	negative	
Phenylpyruvic acid	neg.	negative	

Microscopic

Date: 3-6-92
Time: 8:00 A.M.
Priority: Today

		Normal	Units
Mucous			
Crystals	calcium oxalate 1+		
Bacteria	1+		
Epi cells	occ		
WBC	10-15		/hpf
RBC	6-10		/hpf
Tric			/hpf
Sperm			/hpf
Casts	hyaline 1-3 finely granular 4-8		/lpf

3. The test for phenylpyruvic acid would not have been requested. This is a test done routinely only on children under the age of 2 years.

UNIT 6 / HEMATOLOGY

Learning Activity 1: Terms Relating to Hematology

1. complete blood count
2. erythrocyte sedimentation rate
3. hemoglobin S
4. mean corpuscular hemoglobin
5. mean corpuscular hemoglobin concentration
6. mean corpuscular volume
7. mean platelet volume
8. red blood cell, red blood count
9. white blood cell, white blood count
10. micro
11. agranulocyte—white blood cell with few or no cytoplasmic granules
12. granulocyte—white blood cell with distinct granules
13. platelet—small piece of cytoplasm broken off a larger mass; concerned with clotting mechanism
14. RBC—red blood cell; erythrocyte
15. reticulocyte—young red blood cell
16. WBC—white blood cell; leukocyte
17. In the homozygous condition, the genes are identical. In the heterozygous condition, they are not.
18. capillary action
19. cuvettes, hemocytometer
20. Hemoglobin is a substance found in RBC which transports oxygen throughout the body.

Learning Activity 2: Complete Blood Counts and Other Tests

1. the study of blood
2. white blood count, red blood count, hemoglobin, hematocrit, red blood cell indices (MCV, MCH, MCHC), platelet count, differential white count
3. infection or leukemia, if extremely high
4. too few RBCs or too little hemoglobin within the RBCs
5. the percentage of the blood that is made up of RBCs
6. to initiate the clotting process
7. a. electrolyte b. current c. voltage d. size
8. There is a statistical possibility that two cells may pass through the counting aperture at exactly the same time.
9. to monitor patient progress or therapy
10. a. 42 b. 47
11. by blue granules inside the red blood cell when stained with suprovital stain
12. 1,000
13. 2.2%
14. Blow gently to ensure proper mixing.
15. as percentage of each kind of white blood cell

16. granulocytes, agranulocytes
17. a. red cytoplasmic granules
  b. large blue cytoplasmic granules
  c. neutrophils with lengthened, unsegmented nucleus
  d. pink cytoplasm with small, numerous, pink to bluish-black granules
  e. blue cytoplasm, nonsegmented nuclei
  f. gray-blue cytoplasm with lightly stained gramules, convoluted nucleus
18. erythrocytes, platelets
19. a. variation in the size of red blood cells
  b. variation in the shape of red blood cells
  c. variation in the color of red blood cells
20. done automatically along with the CBC; using a Unipette
21. a. manual hematocrit
  b. manual hemoglobin
  c. platelet count, manual cell counts
  d. hemoglobin (manual)
  e. reticulocyte count
  f. sickle-cell anemia test
  g. sedimentation rate
  h. differential cell count

Learning Activity 3: Coagulation Studies

1. 9:1
2. sodium citrate, EDTA, oxalate
3. (1) lavender top (EDTA), (2) red top (no anticoagulant), (3) blue top (citrate)
4. to bind all calcium immediately
5. clots, hemolysis; these would make tests inaccurate
6. choice of anticoagulant, temperature, carbon dioxide content, pH
7. time between the making of a small incision and the moment bleeding stops
8. Capillary Clotting Time
9. changing fibrinogen into fibrin by the use of thrombin and calcium salts
10. a. in tissue fluid
  b. to accelerate coagulation
  c. blood escapes from a vessel
11. clotting ability of combined factors in the plasma
12. treatment of blood clots
13. a. 10–16 sec.
  b. 28–40 sec.
14. a. Patient can bleed (hemorrhage).
  b. Old blood clots may enlarge, or new ones may form.
15. activated partial thromboplastin time
16. the coagulation factors present

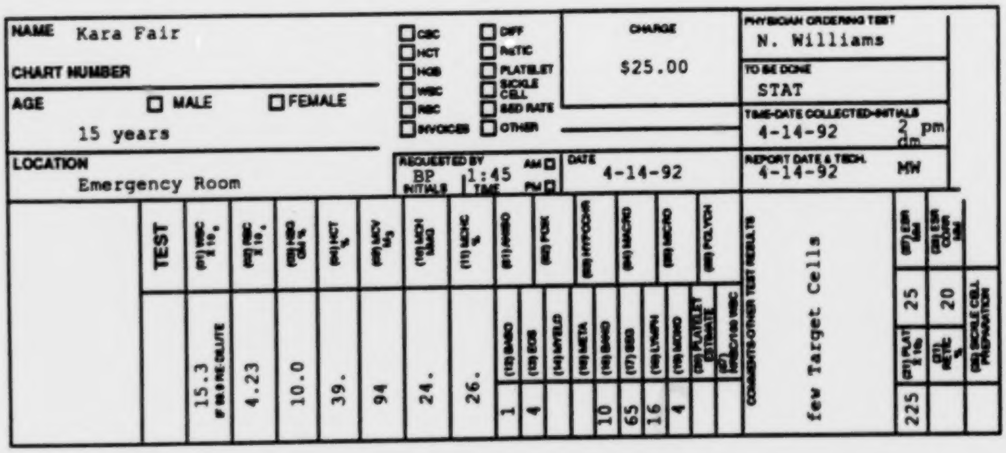
Learning Activity 4: Scrambled Terms

1. hemoglobin
2. ESR
3. homozygous
4. agranulocyte
5. platelet
6. hematocrit
7. capillary action
8. heterozygous
9. reticulocyte
10. hematology

Learning Activity 5: Indices

1. MCV = 88 MCH = 30 MCHC = 35
2. MCV = 87 MCH = 28 MCHC = 32
3. MCV = 97 MCH = 32 MCHC = 34
4. MCV = 87 MCH = 29 MCHC = 34
5. MCV = 122 MCH = 37 MCHC = 31

Learning Activity 6: Hematology Report Forms



NAME Kara Fair

CHART NUMBER

AGE ☐ MALE ☐ FEMALE

15 years

LOCATION

Emergency Room

☐ CBC ☐ DIFF
☐ HCT ☐ RETIC
☐ HGB ☐ PLATELET
☐ WBC ☐ SICKLE CELL
☐ RBC ☐ SED RATE
☐ INVOICES ☐ OTHER

CHARGE $25.00

REQUESTED BY BP **INITIALS** 1:45 **TIME** AM ☐ PM ☐

DATE 4-14-92

PHYSICIAN ORDERING TEST N. Williams

TO BE DONE STAT

TIME-DATE COLLECTED-INITIALS 4-14-92 2 pm dm

REPORT DATE & TECH. 4-14-92 MW

TEST	WBC	RBC	HGB	HCT	MCV	MCH	MCHC
	15.3	4.23	10.0	39.	94	24.	26.

BASO	EOS	MYELO	META	BAND	SEG	LYMPH	MONO	PLATELET ESTIMATE	NRBC/100 WBC
1	4			10	65	16	4		

ANISO	POIK	HYPOCHR	MACRO	MICRO	POLYCH

COMMENTS-OTHER TEST RESULTS few Target Cells

ESR	ESR CORR	PLAT	RETIC %	SICKLE CELL PREPARATION
25	20	225		

Name:	Harry Pratt	**Loc:** Third	**Room:** 306
Patient:	3660892	**Age:** 45	**Sex:** M
Med. Rec. No.:	561189	**Date:** 4-14-92	
Doctor:	R.Romanos	**Time:** 2:30 P.M.	
Diagnosis:			

Hematology

Date: 4-14-92
Time: 2:30 P.M.
Priority: Today

CBC

		Normals Low High	Units
WBC	9.5	4.5-11.0	thous
RBC	4.99	4.6-6.20	mil
Hgb	15.6	14.0-18.0	g/dl
Hct	46.2	40-54	%
MCV	92.6	82-92	fl
MCH	31.3	27-31	pg
MCHC	33.8	32-40	%
Platelets	162,000	150-450	thous
MPV	8.3	7.4-10.4	fl

Diff—Manual

		Normals Low High	Units
Seg Neut	89	50-65	%
Eosino	2	0-3	%
Lympho	5	30-40	%
Mono	4	2-10	%
Poikilocytosis	s/t		
Ovalocytes	few		
Platelets	normal		

Sed Rate

Date: 4-14-92
Time: 2:30 P.M.
Priority: Today

		Normals Low High	Units
Sed Rate	5	0-9	mm/hr

UNIT 7 / SEROLOGY

Learning Activity 1: Terms Relating to Serology

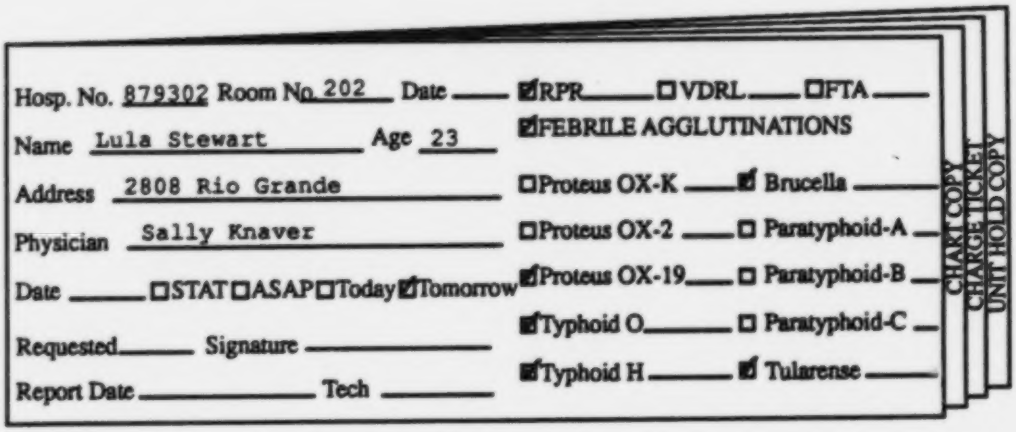
1. acquired immune deficiency syndrome
2. AIDS-related complex
3. enzyme-linked immunosorbent assay
4. fever of unknown origin
5. human chorionic gonadotropin
6. human immune deficiency virus
7. ectopic pregnancy—implantation of fetus outside uterine cavity
8. human chorionic gonadotropin—hormone excreted in the urine of pregnant females
9. hydatiform mole—mole formed by rapid multiplication of chorionic villi to form a mass of cysts resembling a bunch of grapes.
10. agglutination—collection of clumps of cells that are distributed in a fluid
11. flocculation—colloidal phenomenon in which the dispersed phase separates into usually visible particles
12. lysis—destruction of cells by a specific lysin
13. opsonin—substance in blood serum that acts upon microorganisms and other cells
14. precipitation—test reaction during which a deposit falls to the bottom, thus separating out of solution
15. h
16. j
17. l
18. b
19. k
20. m
21. d
22. i
23. a
24. f
25. g
26. c
27. e

Learning Activity 2: Serologic Testing

1. study of antigen-antibody reaction in vitro
2. *In vitro* means that which can be observed in a test tube or on a glass slide as opposed to *in vivo*, which occurs within living organisms.
3. agglutination, flocculation, bacteriolysis, precipitation, complement-fixation, hemolysis, presence of opsonins
4. *Treponema pallidum*
5. Treponemal antibodies are specific and are developed against the spirochete. Nontreponemal antibodies are nonspecific and are developed in response to tissue damage by the spirochetes.
6. FTA-ABS (Fluorescent Treponemal Antibody-Absorption Test), VDRL (Venereal Disease Research Laboratory), RPR (Rapid Plasma Reagin)
7. VDRL, RPR
8. RPR
9. C-reactive protein is an abnormal protein found in persons with infectious and inflammatory conditions.
10. a. within 12 to 24 hours
    b. subsides when the inflammation disappears

11. pneumococcal pneumonia, streptococcal infections, rheumatic fever, lupus erythematosus, sinusitis, pregnancy, use of oral contraceptives, use of an IUD
12. by the ability of the RF factor to agglutinate erythrocytes or latex particles during rapid slide testing
13. No. The following conditions may also yield a positive rheumatoid arthritis test: lupus erythematosus, bacterial endocarditis, cirrhosis of the liver, viral hepatitis, and some forms of leukemia.
14. an enlargement of lymph nodes and spleen, fever, increase in atypical lymphocytes, presence of heterophil antibodies
15. Heterophil antibodies are antibodies that react nonspecifically. For example, these antibodies in the blood of humans may cause the RBC of sheep to agglutinate.
16. to determine the cause of the FUO (fever of unknown origin)
17. typhoid fever, paratyphoid fever, tularemia, undulant fever, typhus fever, trench fever, Rocky Mountain spotted fever
18. specific antibodies formed in response to extracellular products of Group A streptococcus
19. Scarlet fever is caused by a streptococcus. The streptococcus has extracellular products (Streptolysin O antigens) which produce the lysis of RBC (flushed skin color of scarlet fever).
20. antinuclear antibody test (ANA)
21. agglutinins that will cause the person's own RBC to agglutinate at temperatures between 0° and 5°C
22. viral pneumonia
23. a. human chorionic gonadotropin (HCG)
    b. in urine one week after the first missed menstrual period
    c. early pregnancy with a low concentration of HCG
    d. threatened abortion, hydatiform mole, ectopic pregnancy, fetal death
24. ELISA, Western blot test

Learning Activity 3: Completion of a Requisition Form



Hosp. No. 879302 Room No. 202 Date ______ ☑ RPR ______ ☐ VDRL ______ ☐ FTA ______

Name Lula Stewart Age 23 ☑ FEBRILE AGGLUTINATIONS

Address 2808 Rio Grande ☐ Proteus OX-K ______ ☑ Brucella ______

Physician Sally Knaver ☐ Proteus OX-2 ______ ☐ Paratyphoid-A ______

Date ______ ☐ STAT ☐ ASAP ☐ Today ☑ Tomorrow ☑ Proteus OX-19 ______ ☐ Paratyphoid-B ______

☑ Typhoid O ______ ☐ Paratyphoid-C ______

Requested ______ Signature ______

☑ Typhoid H ______ ☑ Tularense ______

Report Date ______ Tech ______

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UNIT HOLD COPY

UNIT 8 / MICROBIOLOGY

Learning Activity 1: Terms Relating to Microbiology

1. medium
   a. broth
   b. agar
2. dermatophyte
3. mycelia
4. filament
5. mycology
6. pathogenic
7. symbiosis
8. bacteriology
9. aerobic
10. anaerobic
11. culture
12. petri dish
13. susceptibility testing
14. optimum-growth temperature
15. normal flora
16. inoculating loop
17. fixing
18. contaminants
19. asepsis

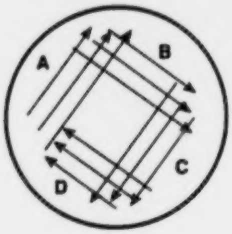
Learning Activity 2: Types of Microorganisms and Their Culture

1. the study of living organisms that can be seen only under a microscope
2. bacteria, yeasts, molds, protozoa, viruses
3. to grow, isolate, and identify the microorganisms causing a disease
4. to prevent overgrowth by contaminants
5. Spinal fluid itself is an excellent medium for growing bacteria.
6. catheterized; midstream, clean catch
7. Swabs from wounds and abscesses suspected of having anaerobes should be placed in an anaerobic transport unit. Swabs for aerobes and yeast should be placed in a sterile transport medium.
8. One must be sure that the bacteria which grow and multiply on the medium are from the patient, not from the medium itself.
9. (1) energy source, (2) carbon, (3) nitrogen, (4) several metallic elements, (5) vitamins and vitaminlike compounds, (6) water
10. broths, nutrient agar, nutrient gelatin, selective media, differential media, enrichment media
11. patient's name, room number, identification number, physician's name, date, time of collection, exact source of specimen
12. so the moisture that forms inside the petri plates will not fall onto the culture
13. aerobic, anaerobic, facultative anaerobic, microaerophilic
14. candle jar, CO_2 incubator, commercial gas packs
15. coccus (round), bacillus (rod-shaped), curved or spiral forms
16. staphylococci, streptococci, diplococci, micrococci
17. spirochetes, vibrios, spirilla

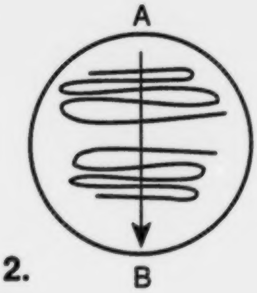
Learning Activity 3: Streaking and Transferring Specimens

1. Remove container top and quickly pass mouth of container over the heating device. Remove swab and roll it along one edge of the petri dish. Place swab in container and replace lid. Heat an inoculating needle. Cool by touching to medium. Streak across areas inoculated by the swab. Heat the needle again and streak across the tip that was just streaked. Heat and repeat. Close petri dish. Heat needle. Store dish.

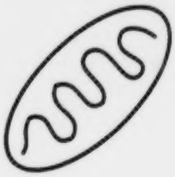
2. Remove lid. Heat mouth of container. Heat loop to redness, touch loop to top of liquid to cool. Touch liquid for transfer. Transfer loopful of liquid to medium. Streak down center of plate. Heat the loop, touch it to the medium to cool, and streak across top half of plate. Turn plate around, and repeat until entire plate is streaked. Cover plate. Flame loop. Store specimen.
3. Remove lid on specimen. Heat mouth of container. Heat loop. Touch slant to cool. Pick up specimen and transfer to surface of the slant, streaking from side-to-side. Replace lid. Flame loop. Store slanted.



1.



2.



3.

Learning Activity 4: Susceptibility Testing and Staining

1. placement of discs containing antibiotics onto a culture of microorganisms to determine which antibiotics would prevent or retard the growth of that organism
2. (a) to ensure even contact so that the antibiotic will diffuse evenly into the medium; (b) so that the discs will not fall off when the plate is incubated upside down
3. (a) amphicillin, carbenicillin, cephalothin, chloramphenicol, gramycin, kanamycin, neomycin, penicillin G, streptomycin, tetracyclin (any five)
   (b) colistin, gantrisin, nolidixic acid, nitrofurantoin, polylmixin B, SXT-TMP (any three)
   (c) bacitracin, clindamycin, erythromycin, methicillin (any two)
4. A broad-spectrum antibiotic is one effective against many types of bacteria.
5. Answers will vary.
6. sensitive (S), resistant (R)

Learning Activity 5: Preparing and Staining Slides

1. Heat an inoculating loop to redness. Cool.
2. a. blue b. red
3. crystal violet, iodine solution, alcohol, safranin
4. It fixes (binds) the stain to bacteria that are Gram-positive.
5. fatty or waxy substances
6. The first stain is heated gently to steaming for 3 to 5 minutes so that the color will become firmly fixed to the fatty/waxy substance in the cell.
7. It clears debris, softens epithelial cells, but leaves the fungal structures intact.
8. to prevent breathing in of spores
9. ringworm, athlete's foot, barber's itch, monilia infections, blastomycosis, histoplasmosis, coccidiomycosis
10. Lacto-Phenol Cotton Blue Stain

11. Flame before and after using, between streaks of a different direction (as when streaking plate for isolation), and between specimens. This procedure prevents contamination.

UNIT 9 / CHEMISTRY

Learning Activity 1: Terms Relating to Chemistry

1. development and use of instruments to complete a testing process without human intervention
2. a. galvanometer—device for measuring electric current.
   b. photoelectric cell—device to detect light and convert it into electricity.
   c. photometer—device that measures light.
   d. spectrophotometer—device that measures light intensities in different parts of the spectrum.
3. a. optical density; light absorbed by a substance during photometry.
   b. physician's office laboratory
4. the portion of light which passes through the substance
5. It is a solution of a known value.
6. to produce changes without being changed itself
7. a. halfway between two extremes
   b. the difference between lowest and highest observations
   c. the ability to repeat a procedure time after time with exactly the same results
   d. results that are steady but on one side of the mean
   e. values that go steadily up or steadily down several days in a row
   f. disagreement of measurements or results but still within acceptable range

Learning Activity 2: Reagents, Water, and Equipment

1. a place in which contents of blood, urine, gastric juice, cerebrospinal fluid, and other body fluids are analyzed to determine whether they are normal or how far they vary from the normal
2. graduated cylinders, volumetric flasks, pipettes
3. Some acids release large quantities of heat when diluted. Adding water can result in sufficient heat to cause boiling and therefore splattering of diluted acid.
4. Store in original container unless different instructions are given on the shipping label or in instructions.
5. distillation, deionization
6. some kind of sampling device, a means to add the reagent and sample, incubating modules (when necessary), modules that measure (usually a colorimeter), a recording device
7. People are still needed to pour the serum into the sample cups to load onto the automated sample tray, and to be aware of the validity of the automated system.
8. size of laboratory, economic situation of laboratory, numbers of tests performed, medical technologist's training and preference
9. colored glass filter, defraction grating

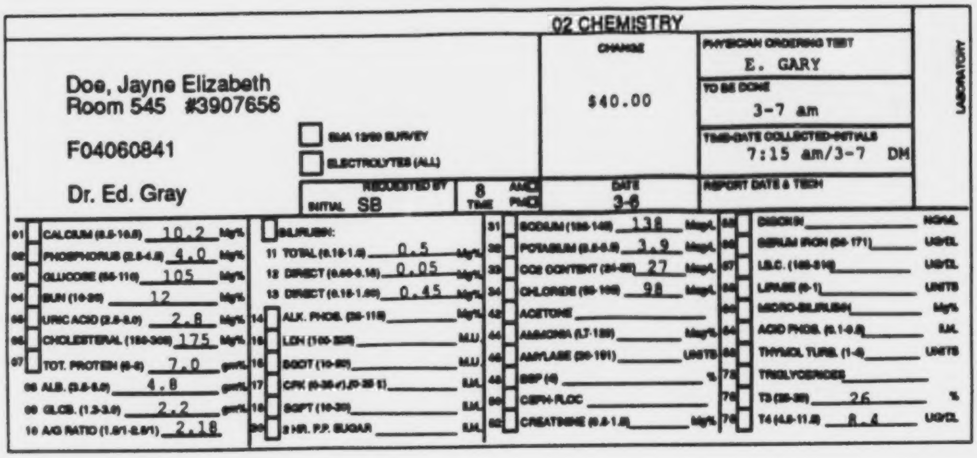
10. a light source, focusing lens, wavelength selector, photocell to convert light to electricity, device to measure the electricity that is produced
11. to use correct wavelength, to use correct selector
12. laboratory procedure carried out in areas such as ICU or OR away from the main lab
13. continuous flow, discrete analysis
14. continuous flow
15. discrete analysis
16. discrete analysis
17. solid-phase analysis

Learning Activity 4: Routine Chemistry Tests

1. Quality control information allows the laboratory to spot potential problems before patient results become meaningless to the physician. Quality control allows the laboratory to take immediate action before a test is completely out of control.
2. equipment serviced and checked regularly; water baths with sufficient water; reagents fresh and uncontaminated; temperature of water baths, heating equipment, and refrigerators where it should be; pH; correctness of wavelengths, techniques (any five)
3. Trends are values that go up and down several days in a row. Shifts are results that are steady but on one side of the mean.
4. Glucose oxidation provides energy for various biochemical processes.
5. a test done after a meal
6. ratio of albumin to globulin in total protein, BUN, uric acid, creatine, creatinine
7. ratio of albumin to globulin in total protein, bilirubin, BSP
8. triglycerides, fatty acids, cholesterol, phospholipids
9. for blood coagulation and kidney function
10. chloride, sodium, potassium, carbon dioxide
11. The HDL is considered "good" because it is a high-density lipoprotein and can be broken down in the liver. The LDL is a low-density lipoprotein stored as fat and considered "bad."
12. The range is 7.35 to 7.45. Deviations outside this range result in acidosis, alkalosis, or even death.
13. a. Amylase breaks down starches.
    b. Lipase breaks down lipids.
    c. Alkaline phosphatase is elevated in obstructive jaundice.
    d. Acid phosphatase is elevated in metastatic prostate cancer.
    e. SGOT/AST is present in heart muscle damage.
    f. SGPT/ALT is found in damaged liver tissue.
    g. LDH is used to diagnose cardiac and liver disorders.
    h. CPK is used in diagnosis and prognosis following heart attacks.
14. a combination of tests to study a specific organ or function
15. the movement of charged colloidal particles through an electrical field
16. because of differences in size, shape, and the electrical charge they carry
17. LDH, hemoglobin
18. therapeutic drug level (must not go too low or too high), drugs of abuse, suicide attempts involving overdoses, and accidental ingestion of drugs by children

Learning Activity 4: Quality Control—Variability
Demonstrated to the satisfaction of the instructor.

Learning Activity 5: Reporting Chemistry Results



02 CHEMISTRY

Doe, Jayne Elizabeth
Room 545 #3907656

F04060841

Dr. Ed. Gray

☐ SMA 12/60 SURVEY
☐ ELECTROLYTES (ALL)

REQUESTED BY INITIAL SB
8 TIME AM PM
DATE 3-6

CHARGE $40.00

PHYSICIAN ORDERING TEST E. GARY
TO BE DONE 3-7 am
TIME-DATE COLLECTED-INITIALS 7:15 am/3-7 DM
REPORT DATE & TECH

LABORATORY

01 ☐ CALCIUM (8.5-10.5) 10.2 Mg%
02 ☐ PHOSPHORUS (2.5-4.5) 4.0 Mg%
03 ☐ GLUCOSE (65-110) 105 Mg%
04 ☐ BUN (10-20) 12 Mg%
05 ☐ URIC ACID (2.5-8.0) 2.8 Mg%
06 ☐ CHOLESTERAL (150-300) 175 Mg%
07 ☐ TOT. PROTEIN (6-8) 7.0 gm%
08 ALB. (3.5-5.0) 4.8 gm%
09 GLOB. (1.5-3.0) 2.2 gm%
10 A/G RATIO (1.0/1-2.0/1) 2.18

☐ BILIRUBIN:
11 TOTAL (0.15-1.0) 0.5 Mg%
12 DIRECT (0.00-0.15) 0.05 Mg%
13 DIRECT (0.15-1.00) 0.45 Mg%
14 ☐ ALK. PHOS. (30-115) ____ Mg%
15 ☐ LDH (100-225) ____ M.U.
16 ☐ SGOT (10-50) ____ M.U.
17 ☐ CPK (0-30♂)(0-20♀) ____ I.U.
18 ☐ SGPT (10-30) ____ I.U.
20 ☐ 2 HR. P.P. SUGAR ____ I.U.

31 ☐ SODIUM (135-145) 138 Meq/L
32 ☐ POTASSIUM (3.5-5.0) 3.9 Meq/L
33 ☐ CO2 CONTENT (24-32) 27 Meq/L
34 ☐ CHLORIDE (95-105) 98 Meq/L
42 ☐ ACETONE ____
44 ☐ AMMONIA (LT-150) ____ Mg%
46 ☐ AMYLASE (30-191) ____ UNITS
48 ☐ BSP (4) ____ %
50 ☐ CEPH-FLOC ____
52 ☐ CREATININE (0.5-1.5) ____ Mg%

☐ DIGOXIN ____ NG/ML
☐ SERUM IRON (50-171) ____ UG/DL
☐ I.B.C. (145-315) ____ UG/DL
☐ LIPASE (0-1) ____ UNITS
☐ MICRO-BILIRUBIN ____ Mg%
☐ ACID PHOS. (0.1-0.5) ____ I.U.
☐ THYMOL TURB. (1-4) ____ UNITS
75 ☐ TRIGLYCERIDES ____
76 ☐ T3 (25-35) 26 %
78 ☐ T4 (4.5-11.5) 8.4 UG/DL

UNIT 10 / THE BLOOD BANK

Learning Activity 1: Terms Relating to the Blood Bank
1. b
2. f
3. i
4. m
5. a
6. g
7. k
8. c
9. h
10. j
11. d
12. l
13. e

Learning Activity 2: Drawing and Processing Blood
1. to replace blood lost through hemorrhage due to trauma or surgery, destruction of erythrocytes, or the body's inability to provide its own blood supply
2. at least 17 years old and not over 65 years
3. Rejection means that a person can never give blood due to conditions such as viral hepatitis; drug or alcohol addiction; malaria; AIDS; tuberculosis; syphilis; diseases of heart, liver, or kidney; convulsive disorders; cancer; blood diseases; and diabetes. Anyone deferred must wait for a certain time period before he/she may give blood. Examples are those receiving transfusions, recent major surgery, tattooing, pregnancy, and vaccinations or immunizations.
4. weigh at least 110 lbs., have normal vital signs, have normal hemoglobin and hematocrit
5. clean with soap and 70% alcohol, apply an iodophore
6. citrate-dextrose solution (ACD)
7. 450–500 cc blood

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8. a special tube of blood collected after the needle is withdrawn from the arm by inserting the needle into a test tube; used for testing done on unit of blood
9. by heat-sealing segments of the collection tubing which can be detached
10. pilot tube, segments of tubing, patient records, blood bank records
11. 1° to 6°C
12. liver disease, hepatitis, HIV virus, syphilis
13. blood group, Rh factor, type of anticoagulant present, donor
14. daily
15. yes, autologous

Learning Activity 3: Blood Groups and Cross Matching

1. Antigens react with a specific substance to produce its specific antibody.
2. Antigens are located on the surface of the red blood cells and are called agglutinogens. Antibodies are located in the serum and are called agglutinins.
3. the antigens or agglutinogens on the surface of the red blood cell
4. two, A and B
5. Anti-B
6. Blood type is hereditary and does not change.
7. antigen, on red blood cells
8. when Rh-negative person receives a transfusion or an Rh-negative woman becomes pregnant
9. If an Rh-negative person receives Rh-positive transfusion, that person may become sensitized and develop antibodies. Further transfusions of Rh-negative blood would cause clumping within the person's blood.
10. The mother is Rh-negative, and the baby is Rh-positive. An antigen-antibody reaction occurs between the infant's red blood cells and the anti-Rh antibodies in the mother's blood. This causes the infant's red blood cells to hemolyze, and the baby becomes anemic.
11. If an Rh-negative mother delivers an Rh-positive baby but has no anti-Rh antibodies, she is given anti-Rh immune globulin during the first 72 hours after delivery.
12. red blood cells, fresh frozen plasma, cryoprecipitate, fresh plasma, dried plasma, gamma globulin, serum albumin
13. incubation at room temperature with saline suspension of blood cells, high protein incubated with cells and serum at 37°C, Coombs test
14. to detect weak or incomplete antibodies
15. that a cross match was done, the time and date for release of the unit, personnel releasing it, and the person receiving the blood

Learning Activity 4: Blood Bank Inventory

The chart should be completed to the satisfaction of the instructor.

UNIT 11 / HISTOLOGY/CYTOLOGY

Learning Activity 1: Terms Relating to Histology / Cytology

1. e
2. b
3. m

8. h
9. g
10. a

4. g
5. l
6. k
7. c
11. j
12. f
13. i
14. d

Learning Activity 2: Receiving, Processing, and Storing Tissue Slides

1. autopsy, surgery, examination
2. smears, liquids
3. The pathologist studies large sections of tissue or whole organs, describes conditions, and indicates parts of study.
4. embedding, blocking, cutting or sectioning, staining, clearing, and mounting
5. 2 cm square and 0.4–0.5 cm thick
6. allowing an embedding medium to penetrate the tissue for support
7. The tissue is fastened to a holder and cut by a knife blade as the microtome wheel is turned.
8. Nicks will show on the section when it is examined microscopically if the knife is not sharp.
9. series of paraffin slices 6–8 inches long joined together by sectioning
10. hematoxylin and eosin
    Hematoxylin stains the nucleus a dark blue color.
    Eosin colors cytoplasm a rosy-red color.
11. a second dye applied to add contrasting color to bring out differences in structures
12. yes
13. peroxidase reaction, PAS, Sudan Black B, Prussian Blue stain
14. a. making microscopic preparations transparent
    b. applying a mounting medium and cover slip
15. screening for cancer
16. cardboard trays for temporary storage, wooden or plastic boxes storing up to 100 slides, permanent storage in cabinets
17. Tissue and patient must be identified, and number must be applied in such a way that tissue carries it through the entire process without loss or confusion.
18. lost during processing
19. to wear gloves, masks, protective eyeware, gowns, and waterproof aprons; to decontaminate with chemical germicide all instruments and surfaces which were contaminated during the procedure
20. Listing should meet with the instructor's approval.

Tests

TEST / UNIT 1

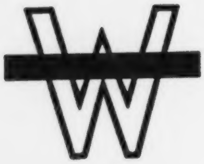
Safety

Directions: Answer the following questions completely.

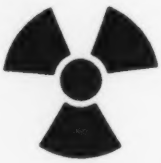
1. Give the meaning of the abbreviation OSHA, and explain the function of this organization.
2. Name five body fluids which require observance of Universal Blood and Body Fluid Precautions during handling.
3. Name the three classes of fires and indicate what flammable materials are included in each class.
4. Name two types of fire extinguishers and on what materials each can be used.
5. Why are codes used in a hospital?
6. List the four codes and what each indicates.
7. List four items of laboratory equipment provided for the workers' safety.
8. Describe a lab worker properly clothed for routine blood work.
9. a. How are accidents reported?

   b. To whom are they reported

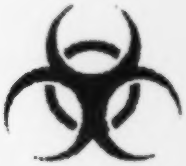
   c. What is included in a report?
10. Identify the following emblems.



a. ____________



b. ____________



c. ____________

TEST / UNIT 2

Organization of the Laboratory

Directions: Answer the following questions.

1. List in order the degrees a person might acquire by continuing his or her education from a junior or community college through the highest degree offered by a university. You may list the abbreviations or write out the names of the degrees.

2. The laboratory worker who heads the laboratory is called the ______________.

3. The medical person in charge of the laboratory is called the ______________ and is a/an ______________.

4. Differentiate between a medical technologist and a medical technician as to education and functions.

5. Differentiate between a cytotechnologist, the histotechnologist, and a histologic technician as to education and functions.

6. How many years of college preparation are required for medical laboratory aides?

7. List five departments within the laboratory and explain the functions performed by each.

8. Define the following terms:
   a. certification

   b. licensure

   c. registry

TEST / UNIT 3

Collecting and Processing Specimens

Directions: Circle the answer which best completes the statement.

1. If a blood sample contains an anticoagulant, the liquid will separate into
   a. serum   b. plasma   c. precipitate   d. hemolysis

2. Cutting off a piece of bone or body structure is called
   a. resection   b. biopsy   c. venipuncture   d. aspiration

3. Abnormal amount of fat appearing in the blood is called
   a. hemolysis   b. anticoagulant   c. lipemia   d. jaundice

4. Puncture of a vein for withdrawing blood is called
   a resection   b. biopsy   c. aspiration   d. venipuncture

5. A relationship marked by harmony is
   a. hostile   b. rapport   c. apprehensive   d. required

6. A substance that prevents blood from clotting is
   a. plasma   b. serum   c. a coagulant   d. an anticoagulant

7. A tube put into the body to withdraw fluids is a
   a. tourniquet   b. bevel   c. catheter   d. lancet

8. The slanted tip of a needle is called the
   a. bevel   b. lancet   c. knife edge   d. gauge

9. Which layer of the blood would **not** be found at the top after sedimentation?
   a. serum   b. plasma   c. supernatant   d. sediment

10. Capillary blood is collected by a
    a. venipuncture   b. lancet   c. biopsy   d. resection

11. Explain the following terms.
    a. logging a specimen

    b. processing a specimen

12. Name four possible sites for collecting capillary blood.

13. List three types of tests performed on feces.

14. What is a routine urinalysis? At what time is the specimen collected?

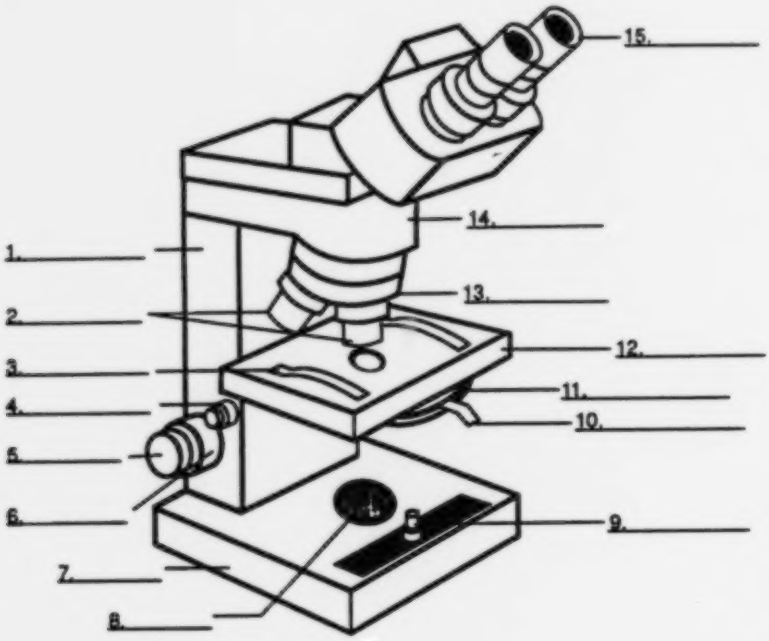
15. State three reasons for collecting specimens for testing.

16. Describe either the procedure for a venipuncture using a syringe and needle or the collection of capillary blood from a finger.

TEST / UNIT 4

Equipment

Directions: Identify parts of a microscope as indicated. Give complete answers to the questions.



16. If an eyepiece has 20X marked on it, what does this mean?

17. Explain what a meniscus is and how it is used.

18. For what two purposes are pipettes used?

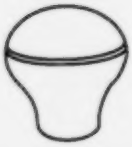
19. Differentiate between volumetric and serological pipettes.

20. Describe the procedure for transferring liquids using a pipette.

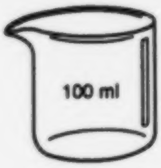
21. Explain how centrifuges are used to separate substances.

22. Name two pieces of equipment which may be used for sterilization.

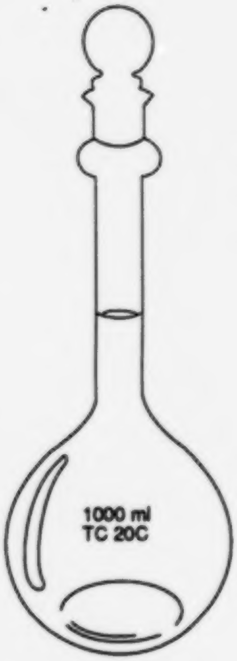
Directions: Identify each of the following pieces of equipment by writing its name in the blank.



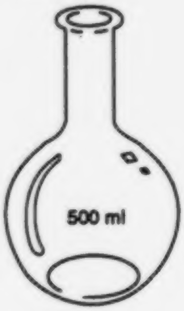
23. ____________



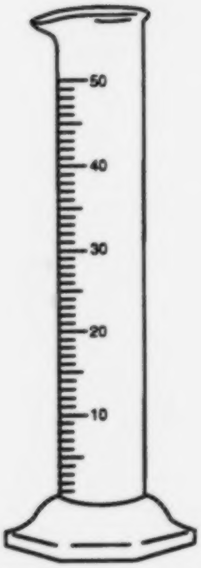
24. ____________


1000 ml
TC 20C

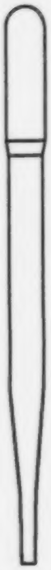
25. ____________



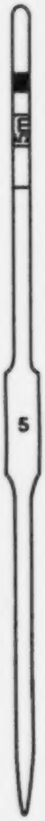
26. ____________


50
40
30
20
10

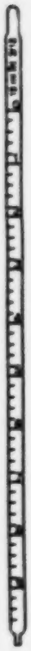
27. ____________



28. ____________



29. ____________



30. ____________

TEST / UNIT 5

Urinalysis

Directions: Answer the following questions completely.

1. a. In a hospital, who is responsible for collecting most of the urine specimens?

   b. In a clinic, who collects most of the urine specimens?

2. Why should examinations be carried out on fresh urine specimens?

3. Name three items included on clinic laboratory labels for urine specimens.

4. What is included in a macroscopic examination?

5. Match each description to a urine color

___ black
___ straw
___ green
___ colorless
___ yellow

a. dye
b. very dilute
c. almost colorless
d. iron, melanin
e. concentrated

6. Name three things that might cause cloudiness in urine samples.

7. What does *pH* mean?

8. Name two methods used to check specific gravity.

9. List the steps to follow in preparing a urine specimen for microscopic examination.

10. Why would you use Sternheimer or crystal-violet safranin stain?

11. List the microscopic structures reported as
    a. average/lpf

    b. average/hpf

12. What is *Trichomonas?* Is it normally found in urine?

13. Define *cast*, and list three types.

14. Listed below are urine crystals. Write in the blank whether each is found in *acid* or *alkaline* urine.

_________________ a. amorphous phosphates
_________________ b. ammonium biurate
_________________ c. uric acid
_________________ d. calcium carbonate
_________________ e. triple phosphate
_________________ f. amorphous urates

15. Name two artifacts that may be seen in urine specimens.

TEST / UNIT 6

Hematology

Part I—Abbreviations and Definitions

Directions: Match the definition to the abbreviation by placing the appropriate letter in the blank.

____ 1. APTT

____ 2. CBC

____ 3. diff

____ 4. EDTA

____ 5. ESR

____ 6. Hb

____ 7. HbS

____ 8. Hmt

____ 9. lymphs

____ 10. MCH

____ 11. MCHC

____ 12. MCV

____ 13. Plt

____ 14. pro time

____ 15. RBC

a. an anticoagulant used in vacuum tubes

b. a young red blood cell

c. a type of granulocyte

d. a test used during the treatment of blood clots

e. leukocyte; serves as body's defense for combating bacteria

f. rate at which red cells settle out in a specified time due to changes in their surface charges

g. chemical, found in red cells, which transports oxygen throughout body

h. abnormal form of hemoglobin which alters oxygen-carrying ability due to change in form of the cell

i. measurement of the percentage of blood made up by red blood cells

j. small piece of cytoplasm that has broken off and is concerned with clotting

k. erythrocyte; contains hemoglobin

l. series of hematology tests routinely done at a particular institution

m. average volume of the red blood cell

n. average weight of hemoglobin in the RBC

o. a screening test for coagulation disorders

_____ 16. retic

_____ 17. WBC

p. weight of hemoglobin per volume of cells

q. report of percentage and kind of leukocytes

Part II

Directions: Answer the following questions.

1. What is *hematology?*
2. List tests included in a complete blood count.
3. What does a high white cell count indicate?
4. What is indicated if someone is anemic?
5. In performing a reticulocyte count, (a) how many red blood cells must be counted and (b) how is the count calculated and reported?
6. What are the two general types of leukocytes?
7. List two examples of each type of leukocyte.
8. Besides reporting the number and kinds of leukocytes, what else must be commented upon in a differential report?
9. The following terms are used to describe red blood cells. To what does each refer?
    a. anisocytosis
    b. poikilocytosis
    c. polychromatophilia
10. Identify:
    a. capillary tubes
    b. cuvette
    c. hemocytometer
    d. Unipette
    e. Sicklequik

Part III—Coagulation

Directions: Answer the following questions.

1. Name two anticoagulants.
2. For what two conditions must all coagulation study tubes be checked?
3. What is meant by *bleeding time?*
4. Of what does coagulation consist?
5. Explain the purpose of these factors in coagulation:
   a. platelets
   b. thromboplastin
6. Name the condition for which the prothrombin time is most useful.
7. Explain what happens when the prothrombin time is lengthened excessively.
8. Explain what may happen when the prothrombin time is allowed to return to normal during treatment.
9. Identify the single most useful procedure for screening of coagulation disorders.

TEST / UNIT 7

Serology

Directions: Give complete answers to the following questions.

1. What is *serology?*

2. Name the organism that causes syphilis.

3. Name the most frequently used test for the detection of syphilis.

4. a. What is C-reactive protein?

   b. How soon after onset of a condition can it be demonstrated?

   c. How soon does it subside?

   d. Name three conditions in which a CRP test may be positive.

5. Which disease is indicated by the presence of a macroglobulin antibody called RF?

6. Is the RF test for the foregoing disease specific? Explain your answer.

7. Name four characteristics of infectious mononucleosis.

8. What is the purpose of febrile agglutination tests?

9. The battery of tests that make up a febrile agglutination can determine the presence of several pathogenic organisms. Name three of these organisms.

10. What causes the flushed skin often found in scarlet fever?

11. What does the ASO test indicate?

12. What disease may be confirmed by a positive cold agglutination?

13. Name two tests used to screen and confirm AIDS.

14. Give the meaning for the following abbreviations:

    a. AIDS

    b. ARC

    c. ELISA

d. FUO

e. HGC

f. HIV

15. Give the meaning of the following terms:

    a. *in vitro*

    b. lysis

    c. precipitation

    d. ectopic pregnancy

TEST / UNIT 8

Microbiology

Directions: In the blanks below, write the term that is defined. Then answer the questions.

aerobic
anaerobic
aseptic
fixed

inoculation
medium
normal flora
pathogenic

1. the introduction of microorganisms onto culture medium 1. ________
2. capable of producing disease 2. ________
3. grow in the presence of oxygen 3. ________
4. to fasten or hold firm; will not wash off with water or staining solution 4. ________
5. free of microorganisms 5. ________
6. organisms usually present but not causing disease 6. ________
7. substance used for the growth of microorganisms 7. ________
8. Explain what will be studied in each of these disciplines:
    a. bacteriology
    b. microbiology
    c. mycology
9. If a specimen cannot be processed within an hour or two after collecting, what should generally be done to preserve it?
10. Which specimen should not be refrigerated?
11. Name four nutritional requirements of bacteria.
12. Name four general types of media.
13. Why are petri dishes containing culture media stored upside down?
14. List three pieces of equipment used to grow anaerobic bacteria.

15. List the three basic shapes of bacteria.

16. Why is it necessary to press the antibiotic discs onto the culture for susceptibility testing?

17. What is the purpose of the iodine in a Gram-staining procedure?

18. What is unusual about the procedure for aid-fast stain?

19. What is the purpose of the 10% KOH in a fungus slide preparation?

20. When should you flame the inoculating loop/needle?

TEST / UNIT 9

Chemistry

Directions: Answer the following questions completely.

1. Name two ways water can be purified for laboratory use.

2. What is the development and utilization of instrumentation that can complete a testing process without any human intervention called?

3. Even in automated laboratory systems, the human element continues to be present. Explain.

4. Colored glass filters and defraction gratings are found in photometers. Both are examples of what portion of a photometer?

5. Differentiate between optical density and percentage of transmittance.

6. Name and describe the two types of technologies utilized in automated clinical laboratory equipment.

7. List at least three things that must be checked to ensure quality control standards.

8. Explain the difference between *trends* and *shifts*.

9. Define these terms:

   a. range

   b. mean

10. Name the test often used as part of routine physical examinations and health screening programs. The oxidation of the substance tested provides energy for various biochemical processes.

11. Abnormal findings on the BUN, uric acid, creatine, creatinine, and the ratio of albumin to globulin may all be indictors of what type of disease?

12. Triglycerides, fatty acids, cholesterol, and phospholipids are examples of what?

13. Name two substances important in muscle and kidney function and in proper blood coagulation.

14. If a physician orders a bilirubin, BSP, and A/G ration, he or she may suspect that the patient has problems with which organ?

15. Name the four tests included in a set of electrolytes.

16. What is the name for the biochemical catalysts that allow the body to carry out its chemical reactions at a moderate temperature and a pH compatible with life?

Directions: Write True or False in each blank.

17. Never add water to acid. 17. ________

18. Reproducibility is when measurements or results disagree but are still within acceptable range. 18. ________

19. Variability means that the same results are obtained today as were obtained yesterday. 19. ________

20. Quality control allows laboratories to spot potential problems before results become meaningless to the physician and take immediate action before a test is completely out of control. 20. ________

TEST / UNIT 10

The Blood Bank

Directions: Answer the following questions.

1. Summarize what happens in a blood bank.
2. Why are transfusions necessary?
3. Supply the terms to match the following definitions:
    a. the person who gives blood
    b. the person receiving a transfusion
    c. a person who supplies blood for his/her own transfusion
4. Give the donor requirements in regard to:
    a. age
    b. weight
    c. vital signs
    d. hemoglobin and hematocrit
    e. AIDS
    f. syphilis
    g. tattooing
    h. pregnancy
5. Describe how a person's arm is prepared for a blood donation.
6. Name the anticoagulant most commonly used in the collecting unit.
7. How much blood is placed into a collecting unit?
8. At what temperature is blood stored?
9. How often is the stored blood inspected?
10. Name the four blood groups.

11. What determines blood type?
12. At what age is the blood type established?
13. Is the Rh factor an antigen or an antibody, and where is it located?
14. Name two occasions when the Rh factor is important.
15. Identify these terms:
    a. plasma
    b. cryoprecipitate
    c. components

TEST / UNIT 11

Histology/Cytology

Directions: Match the terms to the definitions, and write the proper letters in the blanks.

____ 1. cells that have been shed from the body

____ 2. living tissue removed for microscopic diagnosis

____ 3. identification of tissue by its color reaction to dyes

____ 4. solution to prevent decomposition of tissue

____ 5. autopsy

____ 6. screening test for cancer

____ 7. study of cells

____ 8. study of tissue

____ 9. solution to preserve tissue

____10. examination of a dead body

____11. to place tissue sections on slides

____12. tissue frozen for immediate sectioning, staining, and diagnosis

____ 13. allowing embedding medium to penetrate the tissue for support

____ 14. machine with a knife for cutting issue in thin slices

____ 15. removal of water

a. autopsy
b. biopsy
c. cytology
d. dehydration
e. exfoliate cells
f. frozen section
g. fixative
h. histology
i. infiltration
j. microtome
k. mount
l. Papanicolaou smear
m. postmortem
n. staining

Directions: Answer the following questions.

16. Name three occasions when tissues are obtained for examination.

17. In what other forms, besides tissues, do specimens arrive in the histology laboratory?

18. List the main steps in the process of preparing and placing tissues on slides.

19. Cite the two most commonly used dyes in pathology.

20. What is a counterstain?

Answers to Tests

TEST / UNIT 1
Safety

1. Occupational Safety and Health Act, to ensure the safety and health of all workers in the country
2. blood, sputum, semen, vaginal secretions, urine, stool, tissues, drainage from open wounds (any five)
3. A—ordinary combustibles like wood, paper, and cloth; B—flammable liquids, grease, paint, and oils; C—electrical equipment
4. foam—ordinary combustibles, flammable liquids
   carbon dioxide—flammable liquids, electrical equipment
   soda acid—ordinary combustible materials
   pump type—ordinary combustible materials
   gas cartridge—ordinary combustible materials
   multipurpose dry chemical—any flammable material
   ordinary dry chemical—flammable liquids, electrical equipment
5. to indicate that an emergency has occurred and to prevent frightening patients and visitors
6. Code Red—fire
   Code Blue—someone has stopped breathing or the heart has stopped, or both
   Code Yellow—uncontrolled person or a threatening situation in facility
   Code Green—special team of people to report to emergency area
7. fire blanket, safety shower, eye wash, hoods
8. in lab jacket or coat, and gloved
9. a. incident report b. superior c. time, place, cause of accident, witnesses
10. a. do not use water b. biohazard c. radioactive

TEST / UNIT 2
Organization of the Laboratory

1. A.D., B.S. or B.A., M.S. or M.A., Ph.D. or M.D.
2. chief technologist
3. laboratory director, doctor or M.D.
4. The M.T. has a bachelor's degree and one year of training at a teaching hospital. The M.T. is highly trained to carry out complex laboratory procedures. The M.L.T. has completed two years of training at a junior or community college. The M.L.T. performs basic laboratory procedures and routine tests. Both have passed an examination.
5. The cytotechnologist prepares and studies slides of human cells. The C.T. has 2 years of college study and 1 year at a special school. The histotechnologist has studied 4 years at a college and 1 year at a specialized school. The histologic technician has worked 2 years in a pathology laboratory or 1 year in an accredited program. Certification is available for both. Both prepare and preserve body tissues and cells on slides for study.
6. none

7. *bacteriology*—identify bacteria through slides and cultures; run drug sensitivity tests
   blood bank—screen donors, draw blood for transfusion, cross match blood, and investigate transfusion reactions
   chemistry—analyze contents of blood, urine, gastric juice, cerebrospinal fluid, and any body fluid
   cytology—prepare body cells for staining and study on slides
   histology—prepare body tissues for staining and study on slides
   hematology—examine blood cells for abnormalities and study blood coagulation
   parasitology—tests for presence of parasites
   serology—use antigens to test for presence of specific antibodies in tissue or blood; do pregnancy testing
   urinalysis—examine urine for physical, chemical, and microscopic qualities (any five)
8. a. issuance of a certificate by a regulating professional organization attesting that a person has fulfilled standards of education and performance
   b. permission to practice awarded by a state or national board to persons who have proved competency for an occupation
   c. list of qualified individuals kept on an official record, indicating satisfactory completion of standards for some profession

TEST / UNIT 3
Collecting and Processing Specimens

1. b
2. a
3. c
4. d
5. b
6. d
7. c
8. a
9. d
10. b
11. a. recording
    b. preparation of the specimen for analysis
12. finger, earlobe, toe, heel
13. gross examination for consistency, color, mucus, or blood; occult blood; detection of parasites and ova; viral and bacterial cultures; detection of amoeba (any three)
14. series of tests selected by an institution to be performed on most urine specimens, any time
15. to diagnose disease, to determine treatment, to define progress of treatment
16. *Venipuncture:* Place a tourniquet above the site, tying so that it can be released with one hand. Insert the needle cleanly and rapidly with the bevel pointing upward. Aspirate blood. Place a sponge over the puncture site, but do not apply pressure until the needle is withdrawn from the vein. Apply firm pressure, and flex arm until bleeding stops.
    Capillary collection: Select site to puncture. Cleanse with a disinfectant sponge and wipe with dry sponge. Grasp finger across the middle or first joint and puncture skin with lancet. Wipe off first drop of blood and collect with a continuous flow into containers. Prepare blood smears if required and apply pressure with a dry sponge.

TEST / UNIT 4
Equipment

1. carrying arm
2. objectives
3. slide clip
4. condenser adjustment wheel
5. fine adjustment wheel
6. coarse adjustment wheel
7. microscope base
8. substage light
9. switch
10. iris diaphragm lever
11. diaphragm unit
12. stage
13. nosepiece
14. barrel
15. ocular
16. Object is magnified twenty times by the eyepiece.
17. A crescent-shaped curvature found on the upper surface of a liquid as it is measured. It is placed on the calibration when measuring liquids.
18. measuring and transferring liquids
19. Volumetric pipettes measure a single amount of liquid, while serological pipettes measure different volumes within a given amount.
20. Attach bulb to the mouthpiece, place it in the solution, and use suction to draw the solution up until the meniscus is on the mark. Wipe outside of pipette to dry it. Place tip of pipette against side of receiving vessel. Release suction and allow liquid to drain.
21. The centrifuge spins at high speed to separate materials of different densities. The heavier materials are pulled to the bottom by centrifugal force, leaving the lighter materials and liquids on top.
22. hot air oven, autoclave
23. aspirator bulb
24. beaker
25. volumetric flask
26. Florence flask
27. graduated cylinder
28. transfer pipette
29. volumetric pipette
30. serologic pipette

TEST / UNIT 5
Urinalysis

1. a. nursing department b. laboratory personnel
2. Chemical composition of urine is altered upon standing. Bacteria multiply and leukocytes and casts break down.
3. patient's name, doctor ordering test, date collected, test requested (any three)
4. Those carried out without a microscope, using only the eye for color and character.
5. black—d, a
   straw—c
   green—a
   colorless—b
   yellow—e, d
6. crystals, WBC, RBC
7. whether a specimen is acid or alkaline
8. urometer, refractometer

9. Mix urine by swirling gently. Then pour 10 to 12 ml into a centrifuge tube, and centrifuge 5 minutes at 1,500 to 2,000 rpm. Decant and place upright so remaining fluid can mix with sediment. Tap end of tube gently to thoroughly mix remaining fluid and sediment. Pour a small amount on a clean slide, and cover with a cover slip.
10. to stain sediment to make it easier to see
11. *lpf*—casts, epithelial cells, crystals, amorphous materials
    hpf—RBC, WBC, bacteria
12. vaginal parasite not normally found in urine
13. A cast is a cylindrical structure composed of mucoprotein. It has blunt ends and parallel sides. Types are hyaline, granular, RBC or WBC, fatty, waxy, and renal failure. (any three types)
14. a. alkaline
    b. alkaline
    c. acid
    d. alkaline
    e. alkaline
    f. acid
15. talc, starch

TEST / UNIT 6
Hematology

Part I—Abbreviations and Definitions

1. o
2. l
3. q
4. a
5. f
6. g
7. h
8. i
9. c
10. n
11. p
12. m
13. j
14. d
15. k
16. b
17. e

Part II

1. study of blood
2. white blood cell count, red blood cell count, hemoglobin hematocrit, red blood cell indices (MCV, MCH, MCHC), platelet count differential
3. infection or leukemia if excessively high
4. too few RBC or too little hemoglobin within the RBC
5. (a) 1,000
   (b) divide by 10 and report as % of reticulocytes
6. granulocytes, agranulocytes
7. granulocytes—eosinophils, basophils, neutrophils, bands (any two)
   agranulocytes—lymphocytes, monocytes
8. erythrocytes, platelets

9. a. variation in the size of red blood cells
   b. variation in the shape of red blood cells
   c. variation in the color of red blood cells
10. a. used for manual hematocrit
   b. small glass container used in photometers
   c. glass counting chamber used to count platelets and other blood components
   d. used for platelet counts
   e. used for testing for sickle-cell anemia

Part III—Coagulation

1. sodium citrate, EDTA, sodium oxalate (any two)
2. clots, hemolysis
3. the time between the making of a small incision and the moment bleeding stops
4. changing fibrinogen into fibrin by the use of thrombin and calcium salts
5. a. initiate coagulation
   b. accelerate coagulation
6. treatment of blood clots
7. Patient may hemorrhage.
8. Old clots may enlarge or new clots form.
9. activated partial thromboplastin time

TEST / UNIT 7
Serology

1. study of antigen-antibody reaction *in vitro*
2. *Treponema pallidum*
3. RPR
4. a. A CRP is an abnormal protein found in persons with infections and inflammatory conditions.
   b. CRP appears within 12 to 24 hours after the onset of disease.
   c. CRP subsides when inflammation disappears.
   d. It may be found in any of the folowing: pneumococcal pneumonia, streptococcal infections, rheumatic fever, lupus erythematosus, myocardial infarction, some kinds of carcinoma, sinusitis, pregnancy, women using oral contraceptives, women with an IUD, normal persons in older age groups. (any three of these)
5. rheumatoid arthritis
6. No. It is nonspecific. Other conditions may also yield a positive rheumatoid arthritis test.
7. enlargement of lymph nodes and spleen, fever, increase in atypical lymphocytes, presence of heterophil antibodies
8. Febrile agglutinations are ordered when a patient continues to exhibit a fever for unknown reasons.
9. *B. typhi O, B. typhi H, B. paratyphoid A, B. paratyphoid B, B. paratyphoid C, Proteus OX-K, Proteus OX-19, Brucella, F. tularensis* (any three)
10. Extracellular products of *Streptococcus A* (antigens) produce lysis of red cells.
11. The ASO is a test for the presence of antibodies against Group A Streptococci.
12. viral pneumonia

13. ELISA, Western blot test
14. a. acquired immune deficiency syndrome
    b. AIDS-related complex
    c. enzyme-linked immunosorbent assay
    d. fever of unknown origin
    e. human chorionic gonadotropin
    f. human immune deficiency virus
15. a. *In vitro* means that which can be observed in a test tube or on a glass slide as opposed to *in vivo*, which occurs within living organisms.
    b. destruction of red cells by a specific lysin
    c. test reaction during which a deposit falls to the bottom, thus separating out of solution
    d. implantation of fetus outside uterine cavity

TEST / UNIT 8
Microbiology

1. inoculation
2. pathogenic
3. aerobic
4. fix
5. aseptic
6. normal flora
7. medium
8. a. bacteria
   b. living organisms that can be seen only under a microscope
   c. fungi, yeast, molds
9. Refrigerate at 4°C.
10. spinal fluid (CSF)
11. energy source, carbon, nitrogen, metallic elements, vitamins and vitaminlike compounds, water (any four)
12. broths, nutrient agar, nutrient gelatin, selective media, differential media, enrichment media (any four)
13. to prevent moisture from falling onto the culture, therefore preventing isolation of colonies
14. candle jar, anaerobic jar, CO_2 incubator
15. coccus (round), bacillus (rod-shaped), curved or spiral
16. to ensure even contact so that the antibiotic will diffuse evenly into the medium and so that the discs will not fall off when the plate is incubated upside down
17. It fixes (or binds) the stain to the bacteria that are Gram-positive.
18. The first stain (carbol fuchsin) is heated gently to steaming for 3 to 5 minutes so that the color will become firmly fixed to the fatty/waxy substance in the cell.
19. It clears the debris, softens epithelial cells, but leaves the fungal structures intact.
20. Flame before and after using, between streaks of a different direction (as when streaking plate for isolation), and between specimens.

TEST / UNIT 9
Chemistry

1. distillation, deionization
2. automation
3. People are still needed to pour the serum into the sample cups to load onto the automated sample tray, and to be aware of the validity of the automated system.
4. wavelength selectors
5. The light absorbed by the substance during photometry is the absorbance or optical density. The portion of light which passes through a substance is the percentage of transmittance.
6. continuous flow analysis, discrete analysis
7. equipment serviced and checked regularly; water baths at sufficient levels; reagents fresh and uncontaminated; temperature checked on water baths, heating equipment, and refrigerators; pH; correctness of wavelengths; techniques
8. Trends are values that go steadily up or down several days in a row. Shifts are results that are steady but on one side of the mean.
9. a. Range is the difference in the highest and lowest observations.
   b. The mean is halfway between two extremes.
10. glucose
11. kidney
12. lipids
13. calcium, phosphorus
14. liver
15. sodium, potassium, chloride, carbon dioxide
16. enzymes
17. True
18. False
19. False
20. True

TEST / UNIT 10
The Blood Bank

1. Blood is drawn from donors, tested, labeled, and placed in the bank. Cross matches are done. The blood is issued for transfusions.
2. to replace blood lost through hemorrhage due to trauma or surgery, destruction of erythrocytes, or the body's inability to provide its own blood supply
3. a. donor
   b. recipient
   c. autologous blood donor
4. a. 17 to 65 years of age
   b. over 110 lb
   c. normal
   d. normal
   e. rejected

    f. rejected
    g. defer
    h. defer
5. cleaned with soap and 70% alcohol, iodophor applied
6. citrate-dextrose solution (ACD)
7. 450–500 cc
8. 1°–6°C
9. daily
10. A, B, AB, O
11. the antigens or agglutinogens on the surface of the red blood cells
12. inherited
13. antigen on red blood cell
14. when an Rh-negative person receives a transfusion and when an Rh-negative woman becomes pregnant
15. a. liquid portion of blood
    b. the precipitate formed when plasma is frozen
    c. portions of blood separated from a single unit

TEST / UNIT 11
Histology/Cytology

1. e
2. b
3. n
4. g
5. m
6. l
7. c
8. h
9. g
10. a
11. k
12. f
13. i
14. j
15. d
16. autopsy, surgery, examination
17. smears, liquids
18. embedding, blocking, cutting or sectioning, staining, clearing, and mounting
19. hematoxylin, eosin
20. a second dye applied to add contrasting color to bring out differences

Projects for More Advanced Study

Medical Laboratory

Unit 1 / Safety in the Laboratory

1. Make a drawing of the medical laboratory where you are assigned showing the location of all equipment for use with fires and other emergencies.

2. Ask permission of your supervisor to use the hospital fire plan and the disaster plan to develop a paper showing the role of the laboratory during a disaster. Describe how the laboratory responds to a fire in its area.

Unit 2 / Organization of the Laboratory

1. Devise a work flow chart for the laboratory where you are assigned, if it is small, or for a department in the lab, if the facility is large. Show how a specimen moves through the laboratory from its reception to the final reporting of results.

2. Select two departments within the laboratory, and list all of the tests available in each of these.

Unit 3 / Collecting and Processing Specimens

1. Assume that you are employed in a small laboratory and have just hired an assistant. In order to save time in making explanations, you prepare written instruction for venipuncture, collecting capillary blood, and collecting a stool specimen at home. Develop and write out these three sets of instructions. The formats must be different from those in the Student's Manual.

2. Talk with your supervisor to determine which tests are sent to other labs for testing. List the names of the tests and the other laboratories that perform the testing. The list may include commercial, reference, experimental, or governmental facilities.

Unit 4 / Laboratory Equipment

1. Research and prepare a paper to explain how the dark field microscope, the electron microscope, the ultraviolet microscope, and the phase contrast microscope are used in laboratories. Explain their special adaptations and how these operate. Diagrams may be included to illustrate the structure and operation of this equipment.

2. Make a list by departments of the different types of centrifuges used in the laboratory where you are assigned. Give examples of procedures for which these centrifuges are used. Illustrations of different types can be included. Answers may be in chart form or as an essay.

3. Make a list of all equipment in the place where you are assigned which must be maintained at a constant temperature. Ascertain and record the acceptable temperature range allowed. Take a reading, and record the temperature you find on a specific date.

4. Either calibrate or assist in the calibration of a centrifuge. Describe the procedure in a paper. Record all data.

Unit 5 / Urinalysis

1. Make a series of colored drawings to show cellular elements, casts, and crystals found in acid and in alkaline urine.

2. Write a report on the principle of agglutination inhibition tests for pregnancy. Diagrams may be used to illustrate the reactions.

3. Write a brief report on the etiology and prognosis of *Trichomonas*. Provide a drawing of the parasite.

4. Explain in a research paper how and why dialysis is done. Describe the process and equipment. Illustrations may be included.

Unit 6 / Hematology

1. Conduct research to discover the types, causes, and treatment of anemia. Develop a paper or chart to present your findings.

2. List and describe or draw equipment needed for a manual white cell count. Outline the procedure in a paper, explaining each step.

3. Prepare and stain six slides for differential counts. Do a differential count on each of the six slides. Draw an example of each type of formed element seen. Submit an account of the process for preparing the slides and counting the cells. Submit the drawings and slides with the paper for grading.

4. Research and explain in a paper the etiology and prognosis of sickle-cell anemia. Describe tests used to determine HgS.

5. Develop a list of all of the factors involved in the clotting of blood. Discuss the process of clotting. Describe abnormal conditions resulting from the lack of a factor in your report.

Unit 7 / Serology

1. Discuss the etiology and prognosis of infection with *Treponema pallidum*. Describe all three stages of the disease. In a report, list and explain tests used in its detection.

2. Select three of the sexually transmitted diseases. Describe the cause, signs, and symptoms of each in a report. Explain how each is detected through laboratory testing.

3. Use resources to write a report about arthritis, including symptoms, types, tests used in diagnosis, treatment, and age at onset of disease. An alternative is to interview a friend or acquaintance who has arthritis. Prepare a written case history, including all of the information asked for in the other assignment.

4. Research and prepare a paper on the etiology and prognosis of systemic lupus erythematosus. Include types, symptoms, and tests used to detect the condition.

Unit 8 / Microbiology

1. Compile a list of all media available in microbiology where you are assigned. Explain the purpose or use of each.

2. Prepare six slides of different bacteria. Stain them with Gram's stain. Make a drawing of each. Submit the labeled drawings and slides for grading.

3. Research and prepare a report on any two of these bacteria: *Escherichia coli, Shigella, Salmonella enteritidis, Corynebacterium diphtheriae, Bacillus anthracis, Clostridium tetani,* and *Neisseria meningitides*. Describe the morphology of the organism and what disease it causes.

Unit 9 / Chemistry

1. Discuss the importance of electrolytes in a report. Explain where they are found and what occurs when they increase or decrease beyond normal range. Be sure to include a discussion of pH. Define relevant terms, and give test procedures.

2. Research the diagnosis and prognosis of gout and other diseases with similar etiologies. Describe the chemical tests used for detecting this condition.

3. Determine what quality control is and why it is important. Survey the laboratory where you are assigned to determine what measures are used to assure quality. Devise a chart or listing to show methods of quality control in each section of the lab.

Unit 10 / The Blood Bank

1. Research and write a paper about the genetics involved in the ABO and Rh blood group systems. Drawings may be included.

2. List and discuss other antibodies encountered in cross-matching blood. Describe tests for detecting these.

3. Prepare a complete list of conditions causing a donor to be permanently rejected or deferred. List time restrictions for deferred conditions. State the normal range of acceptance for vital signs and hematocrit.

4. If you are old enough and in good health, donate a unit of blood to the blood bank. Write a paper about your experience. Describe the procedure.

Unit 11 / Histology/Cytology

1. Observe and write a paper to describe how a gross examination of tissue is conducted by a pathologist.

2. Ask your supervisor if any medically significant tissues or slides have been preserved. Write a report telling why these are important enough to save. Be sure to research the condition so that you can describe three of these tissues or slides in a report.

3. List five special stains, and describe the process of staining step by step. State the reasons for using each stain.

4. Make a flowchart to show the steps through which a specific specimen would pass from reception in histology to a finished slide. Be sure to indicate all processes within each step, including methods of identification and records.

Suggested Answers

Projects for More Advanced Study

Unit 1 / Safety in the Laboratory

1. The drawing should include the location of sprinklers, alarms, extinguishers, fire blanket, fire doors, exits for use in case of fire, and any other equipment. The location of equipment for other emergencies should include the eye wash station, safety shower, first aid equipment, and any other emergency equipment peculiar to a facility.

2. The fire plan and disaster plan will vary with the facility. The paper should meet with the approval of the instructor.

Unit 2 / Organization of the Laboratory

1. The work flow chart should show receipt of the specimen, its logging in, preparation, and direction to one or more departments. Within a department, the flow will indicate tests performed and how results are reported. The instructor should determine how many departments to include in the flow chart when a large laboratory is involved.

2. Lists will vary with the facility, but should include those tests generally found in such a department. Use those tests discussed in the Student's Manual as a basis. Additional tests should be added.

Unit 3 / Collecting and Processing Specimens

1. The three procedures will include information in the Student's Manual, but it should be organized into sequential step-by-step instructions.

2. The list will vary with the facility, but should be evaluated by the instructor for completeness.

Unit 4 / Laboratory Equipment

1. The different types and uses of microscopes are summarized below:
   a. *dark field microscope*—has a special condenser which magnifies light, resulting in a light object on a dark background; used to detect spirochetes.
   b. *electron microscope*—used in very large hospitals and research labs to produce a powerful resolution of objects through the use of short wavelength electrons and magnification.
   c. *ultraviolet microscope or fluorescent microscope*—uses ultraviolet light to cause stained organisms to fluoresce for purposes of identification; used to confirm mycobacteria and syphilis.

d. *phase contrast microscope*—used to view transparent, unstained specimens by the contrast between their different densities which affects the amount of light they transmit; used on urine sediment and reticulocytes.

2.

Department	*Type of Centrifuge*	*Procedure*
phlebotomy or collection	table/floor model	separate blood into components
urinalysis	table/floor model	prepare sediment for microscopic exam
hematology	table model for hematocrits	hematocrit
	table/floor model	coagulation studies
blood bank	table/floor model	prepare serum cross matching separate blood into components
	refrigerated	cryoprecipitates
chemistry	table/floor model	varies with procedures
serology	table/floor model	separate serum
	special	varies with procedure
microbiology	table/floor model	varies with procedure

3. The equipment list should include water baths and heating blocks with elevated temperatures; slide view boxes in blood bank at 37°C; refrigerators in general usage; the blood bank refrigerator kept at 1° to 6°C; incubators in microbiology at 37°C; temperature regulation for automated equipment; and heated water baths in the blood bank and hematology.

4. The procedure is described in Unit 4 of the Student's Manual. However, different procedures or modifications are acceptable from local facilities.

Unit 5 / Urinalysis

1. Black-and-white drawings of these are found in the unit. Not all of the elements will show color.

2. The first morning urine sample is usually used for pregnancy testing. The hormone chorionic gonadotropin (HCG) appears in the urine about one week after the first missed menses. In an agglutination inhibition test for pregnancy, the HCG will prevent agglutination, thus giving a positive test. If the HCG is not present, agglutination will occur giving a negative test.

3. *Trichomonas* is a flagellated protozoa parasitic in the vagina. Its presence is accompanied by persistent itching, burning, and a white discharge. A drawing of it is included in the Student's Manual.

4. Dialysis is used following acute and chronic kidney failure. The causes of failure are many. Blood is diffused across a semipermeable membrane to remove toxic waste products, to maintain fluid balance, and to maintain the acid-base balance. Dialysis is used in acute drug intoxication also.

Unit 6 / Hematology

1. Anemia is a symptom, not a disease. It is classified by the red cell indices and other factors relating to etiology. The classifications are (1) excessive blood loss through hemorrhage; (2) blood destruction; and (3) decreased blood formation due to diet, toxic conditions, or disease. Students should discuss causes of each condition, with the treatments required.

2. The drawings should include a white blood cell diluting pipette, aspiration tubing, diluting fluid, pipette shaker or hand rotation, and hemocytometer. A description of the method should include dilution, shaking, loading the hemocytometer, and making and reporting the count.

3. The slides should be prepared to the satisfaction of the instructor. The paper should include a description of the techniques used, similar to those in the Student's Manual for preparing a smear, staining with Wright's stain, and conducting a differential count. The drawings should picture granulocytes, agranulocyte, erythrocytes, and thrombocytes.

4. A discussion of the inheritance of both sickle-cell anemia and sickle-cell trait is required. Two tests (Sicklequik and electrophoresis) are discussed in Unit 6. The paper should include these and any other test used locally.

5. The process of coagulation is discussed in the Student's Manual. In addition to this process, the report should include information about Factors I–V, Factors VII–XIII, what each factor represents, and how it functions. Hemophilia and its inheritance should be included.

Unit 7 / Serology

1. *Treponema pallidum* is the spirochete causing syphilis. Symptoms of the primary, secondary, and tertiary stages should be reported. Testing should include a discussion and description of the early tests (e.g., Wasserman), later tests (e.g., VDRL), those used locally (e.g., RPR), and more advanced confirmation tests (e.g., FTA). Treatment and appearance of resistant strains should be included.

2. Sexually transmitted diseases may include any of the following: AIDS, chlamydia, gonorrhea, herpes, lymphogranuloma venereum, syphilis,

trichomonas. Causes, signs, symptoms, and methods of testing will depend on the three organisms chosen. Each report must be evaluated by the instructor for completeness.

3. Arthritis is an inflammatory joint disease caused by infection, neurological disturbances, degeneration of joints, metabolic disturbances, neoplasms, and other conditions. Testing for the rheumatoid factor is mentioned in the unit. Students should present ways of testing for different types of arthritic conditions and include those tests used in the lab where they are assigned.

4. Lupus erythematosus is a chronic, progressive condition of unknown etiology. It is one of the collagen diseases. The types are cutaneous and systemic. The report should include symptoms of each type and especially mention the butterfly rash. Lupus will give a positive in tests for the rheumatoid factor. The ANA test is used in diagnosis.

Unit 8 / Microbiology

1. This list will vary with the facility.

2. The slides and drawings must be completed to the satisfaction of the instructor.

3. Below is listed brief information about the morphology and pathology of the seven bacteria.
   a. *E. coli*—Gram-negative bacillus causing urinary tract infections, GI upsets, and septicemia
   b. *Shigella*—Gram-negative bacillus causing GI upset and dysentery
   c. *Salmonella enteritidis*—Gram-negative enteric bacillus causing food poisoning and GI upset
   d. *Corynebacterium diphtheriae*—Gram-positive bacillus which causes diphtheria
   e. *Bacillus anthracis*—Gram-positive spore-forming bacillus causing anthrax in animals and septicemia in man
   f. *Clostridium tetani*—anaerobic Gram-positive bacillus causing tetanus
   g. *Neisseria meningitides*—Gram-negative coccus causing meningitis

Unit 9 / Chemistry

1. The term *electrolytes* refers to the charged particles found in the extracellular fluid and within the cellular fluid. The electrolytes consist of two anions, chloride and bicarbonate, and two cations, sodium and potassium. These affect acid-base balance, hydration, pH, osmotic pressure, and the function of heart and muscles. Ions are lost through different mechanisms leading to imbalances, such as acidosis or alkalosis. The pH of the blood is maintained within narrow limits. Students should explain pH, acidosis, alkalosis, the relationship of the bicarbonate to carbon dioxide. Imbalance cannot be tolerated by the body for long. Thus, these tests may be emergency procedures. Local methods of testing should be discussed.

2. Gout is a form of metabolic disease, either hereditary or acquired. Symptoms include inflammation of joints, similar to arthritis, from deposits of sodium citrates around the joints due to faulty purine metabolism. It may be chronic leading to deformities. Reports should discuss occurrences in both sexes, symptoms, and the locations affected. Tests include screening for the rheumatoid factor, specific test for uric acid, and x-rays.

3. Quality control is discussed in the unit. Students should list the departments in the laboratory where assigned, with the control method used by each department. Examples are the positive and negative controls used for RPR testing in serology and standard solutions in chemistry. Routine tests to monitor equipment should be listed. Examples are checking the recording thermometer in the blood bank and checking velocity of centrifuges.

Unit 10 / The Blood Bank

1. This paper should include the inheritance of ABO types—one gene from each parent (AB), lack of either in the case of type O, and the mixed inheritance of OB and AO. Various pairings which appear in offspring should be discussed or shown in diagrams. The same type of inheritance for the Rh factor should be shown. (*Note:* Biology texts are a resource for this assignment.)

2. When unexpected reactions to transfusions occur, testing is conducted for other antigens. The antibodies usually develop following blood transfusions or pregnancy. A few occur naturally. Examples of these antigens are Kell, Duffy, Kidd, MNSs, P, Lutheran, Vel, etc. Some are named for the first patient in whom they were noted. The irregular antibodies appear in incompatible cross matches, making further testing necessary. Students should describe local screening tests used for detection of irregular antibodies.

3. A general discussion of conditions causing a donor to be permanently rejected or deferred is given in Unit 10. Students should devise a new format containing a list of each disease or condition. The list should show permanent rejection or deferment for a specified time. Vital signs and the hematocrit should be listed, with the range of acceptance for each sex.

4. This project is left to the discretion of the instructor and local school policy. The student should describe the full process of screening and drawing a donor. Donations might be incorporated into a HOSA project.

Unit 11 / Histology/Cytology

1, 2. These projects must be evaluated for accuracy and completeness by the instructor.

3. Four stains are listed in the unit—peroxidase reaction, periodic Acid-Shiff stain, Sudan Black B, and Prussian blue stain. Students may select others. Steps in the process of staining should be outlined for each. Students should explain why each is used (e.g., PAS for intracellular glycogen).

4. The flowchart will vary with the facility and must be evaluated by the instructor for accuracy. The chart should show receipt of the specimen, its logging in, preparation, and direction through the department to the final report.

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Competency Chart
for
Medical Laboratory Aide

Directions: Evaluate the trainee, using the rating scale below. Place a check in the appropriate square to indicate degree of skill.

Rating Scale:
4 **Skilled**—can work independently with no supervision
3 **Moderately skilled**—can perform job completely with limited supervision
2 **Limited skill**—requires instruction and close supervision
1 **No exposure**—no experience or knowledge in this area

(1) Identify safety practices

	4	3	2	1
• locate emergency equipment				
• use aseptic techniques				
• wash hands aseptically				
• wear protective clothing				
• dispose of contaminated materials using precautions				

(2) Distinguish work areas

distinguish worker qualifications and responsibilities				

(3) Wash, rinse, and dry glassware and instruments using acceptable methods				
• explain process for cleaning and disinfecting work area				
• use autoclave				
• use oven				
• name types of glassware				

(4) Fill tubes and bottles with specified solutions, and apply identification labels				
• prepare reagents				
• fill tubes with venous blood				
• fill capillary tubes				
• collect urine specimen				
• collect stool specimen				
• label specimen				
• log specimen				

(5) Label and file microscope slides

• prepare and stain slides for differential white counts				
• prepare and stain bacteria with Gram's stain				
• prepare and stain fungi				
• prepare slides for histologic study				
• describe laboratory filing system				
• identify parts of a microscope				

4 3 2 1

(6) Arrange specimens and samples on trays to be placed in incubators and refrigerators
- select temperature range for equipment
- explain nutritional and oxygen requirements for bacteria
- arrange blood by group and Rh for placement in bank

(7) Perform routine tests in medical laboratory under supervision of medical technologist or other qualified individual utilizing prescribed procedures

a. Urinalysis
- prepare specimen
- perform macroscopic examination
- determine specific gravity
- use reagent strips
- conduct microscopic examination

b. Hematology
- identify components of CBC
- distinguish blood cells
- perform counts on blood cells
- perform sickle-cell test
- differentiate between bleeding and clotting times
- explain significance of prothrombin time and APTT

c. Serology
- perform tests for RPR
- C-reactive protein
- infectious mononucleosis
- ASO titer
- cold agglutinins
- pregnancy test
- describe tests for detecting AIDS

d. Microbiology
- list types of growth media
- streak plates
- culture anaerobes
- describe shape of bacteria
- apply antibiotic susceptibility disks

e. Chemistry
- distinguish manual from automated procedures
- list general categories of test procedures
- describe uses of electrophoresis
- discuss therapeutic drug monitoring
- describe how quality is controlled

f. Blood bank
- screen blood donors
- outline process for drawing and evaluating donor blood
- perform type and Rh
- discuss cross match procedures
- list and define blood components

4	3	2	1

g. Histology/cytology
- list specimen sources
- outline steps in processing
- outline steps in staining

Additional competencies

____

____

____

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ASSISTING IN THE MEDICAL LABORATORY

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Assisting in the Medical Laboratory

Student's Manual

By

Helena J. Fair

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COMPLIANCE STATEMENT

TITLE VI, CIVIL RIGHTS ACT OF 1964; THE MODIFIED COURT ORDER, CIVIL ACTION 5281, FEDERAL DISTRICT COURT, EASTERN DISTRICT OF TEXAS, TYLER DIVISION

Reviews of local education agencies pertaining to compliance with Title VI Civil Rights Act of 1964 and with specific requirements of the Modified Court Order, Civil Action No. 5281, Federal District Court, Eastern District of Texas, Tyler Division are conducted periodically by staff representatives of the Texas Education Agency. These reviews cover at least the following policies and practices:

(1) acceptance policies on student transfers from other school districts;

(2) operation of school bus routes or runs on a non-segregated basis;

(3) nondiscrimination in extracurricular activities and the use of school facilities;

(4) nondiscriminatory practices in the hiring, assigning, promoting, paying, demoting, reassigning, or dismissing of faculty and staff members who work with children;

(5) enrollment and assignment of students without discrimination on the basis of race, color, or national origin;

(6) nondiscriminatory practices relating to the use of a student's first language; and

(7) evidence of published procedures for hearing complaints and grievances.

In addition to conducting reviews, the Texas Education Agency staff representatives check complaints of discrimination made by a citizen or citizens residing in a school district where it is alleged discriminatory practices have occurred or are occurring.

Where a violation of Title VI of the Civil Rights Act is found, the findings are reported to the Office for Civil Rights, Department of Health, Education and Welfare.

If there is a direct violation of the Court Order in Civil Action No. 5281 that cannot be cleared through negotiation, the sanctions required by the Court Order are applied.

TITLE VII, CIVIL RIGHTS ACT OF 1964: EXECUTIVE ORDERS 11246 and 11375; TITLE IX 1973 EDUCATION AMENDMENTS: REHABILITATION ACT OF 1973 AS AMENDED; 1974 AMENDMENTS TO THE WAGE-HOUR LAW EXPANDING THE AGE DISCRIMINATION IN EMPLOYMENT ACT OF 1967; VIETNAM ERA VETERANS READJUSTMENT ASSISTANCE ACT OF 1972 AS AMENDED IN 1974.

It is the policy of the Texas Education Agency to comply fully with the nondiscrimination provisions of all federal and state laws and regulation by assuring that no person shall be excluded from consideration for recruitment, selection, appointment, training, promotion, retention, or any other personnel action, or be denied any benefits or participation in any programs of activities which it operates on the grounds of race, religion, color, national origin, sex, handicap, age, or veteran status (except where age, sex, or handicap constitute a bona fide occupational qualification necessary to proper and efficient administration). The Texas Education Agency makes positive efforts to employ and advance in employment all protected groups.

Notes for the Student

Assisting in the Medical Laboratory is designed for use by students investigating the activities within the medical laboratory in a hospital, clinic, or physician's office. The material is designed to relate training experience to information studied in the classroom. Information is provided which is current and relevant. The material is designed for individualized study and is competency-based with educational outcomes stated for each unit. *Assisting in the Medical Laboratory* is made up of three separate publications.

- The **Student's Manual** contains learning objectives, terms and definitions, and information relating to current practices. Topics addressed are the organization, personnel, and types of equipment utilized in laboratories. Collection and processing of specimens is described. Techniques, both manual and automated, are discussed for the following departments: urinalysis, hematology, serology, microbiology, chemistry, the blood bank, and histology/cytology. Safety and quality control are emphasized.
- The **Student Learning Activities** are individualized and designed to encourage the student to carefully read and study the information presented. Each unit has a section for those students desiring more advanced study. Instructors are encouraged to provide one set of activities for each student to facilitate learning.
- The **Instructor's Guide** has a list of essential elements addressed by the units of instruction. A basic test is provided for each unit. Instructors are encouraged to enlarge these. Answers to tests and learning activities are also provided, and a competency chart is included.

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Portions of this book are adapted from two EIMC publications: *Medical Laboratory Assistant* (1981), by Sara Barnett, and *Medical Laboratory Services* (1981), by Catherine Williams.

1

Safety in the Laboratory

Objectives: After completing this unit, you will be able to—

1. *discuss the Occupational Safety and Health Act (OSHA) and the Centers for Disease Control;*
2. *explain procedures for fire prevention, reporting, and control;*
3. *dispose of contaminated materials and equipment using universal precautions;*
4. *follow safety procedures in operating laboratory equipment;*
5. *identify safety practices in the laboratory;*
6. *explain the procedure for reporting accidents; and*
7. *identify the hazards indicated by the National Fire Protection Association emblems.*

Terms and Definitions

CDC—Centers for Disease Control, a federal public health agency with headquarters in Atlanta, Georgia.
code—used to signal an emergency; names of colors may be added to indicate type of problem.
corrosive—a chemical able to corrode or wear away by its action, such as strong acids.
extinguish—to put out or quench; to cease burning.
flammable—easily set on fire.
hazard—danger, risk.
ignite—to set on fire.
incident report—report of an unusual event or accident that does not fit into normal procedure.
incinerate—to burn to ashes.
legible—easy to read.
liability—under legal obligation; responsible for.
NFPA—National Fire Protection Association.
OSHA—Occupational Safety and Health Act; also, Occupational Safety and Health Administration.
safety—freedom from danger, risk, or injury.

Safety means freedom from danger, risk, or injury. The clinical laboratory contains many potential hazards and is constantly concerned with safety. Personnel in the laboratory use hazardous materials such as chemicals, glass, infectious materials, radioactive materials, or compressed gases in their daily work. They use pipettes for transfer, microtome blades for tissue preparation, needles, and corrosive chemicals. The list of potential dangers is a long one. In order to avoid injury and danger to yourself and to others, it is important to always be aware of hazards and to practice safety methods.

Workers in the medical laboratory are carefully trained to deal with potential hazards. While incidents do occur, those which cause serious injury are extremely rare. Disease transmission is minimal.

Regulatory Agencies

In an effort to ensure safe and healthful working conditions for every worker in this country, the federal government has established general safety rules for employers. These are outlined in the Occupational Safety and Health

Act, or OSHA. All employers must conform to OSHA's safety and health standards, which are enforced by the Secretary of Labor. Any worker who feels that conditions on the job are unsafe and do not meet OSHA specifications may ask the local OSHA office to investigate and make recommendations for improvement.

Health care facilities practice Universal Blood and Body Fluid Precautions. Rules are mandated by the Centers for Disease Control, Atlanta, Georgia. All patients and staff are considered possible carriers of harmful pathogens. Body fluids that may be infected include blood, sputum, semen, vaginal secretions, urine, stool, tissues, and drainage from open wounds. In order to decrease the risk of accidental infection, a facility will have strict infection control procedures to be followed by all staff members when handling any body fluid.

Fire Safety

All laboratory personnel must be responsible for fire prevention and control. Most laboratories have the following equipment to control fires: fire doors, fire blankets, and fire extinguishers. A *fire door* is a heavy, fireproof door located between defined areas of a building. In case of fire, the doors are closed to help contain the fire.

A *fire blanket* is used to put out flash fires of clothing as chemical extinguishers may be harmful to the skin. To use a fire blanket, you grasp the rope handle, place the right arm through the rope loop, and pull vigorously while turning to the left. This wraps the person in the blanket as it is unrolled and extinguishes the fire.

Figure 1 shows the three classes of fires and the types of *fire extinguishers* approved for use on that type of fire. Figure 1 also shows how to operate the extinguishers efficiently. Be alert to determine what is burning and to use the correct extinguisher. Using water on burning liquids and electrical wires would spread the fire and provide a means of conducting electricity.

There are three types of fires. Class A fires are those in which the fuel is an ordinary combustible, such as wood, paper, or cloth. Class B fires are those fueled by flammable liquids. Class C fires involve electrical equipment, such as motors and switches. Medical laboratories contain materials that fit into all three categories.

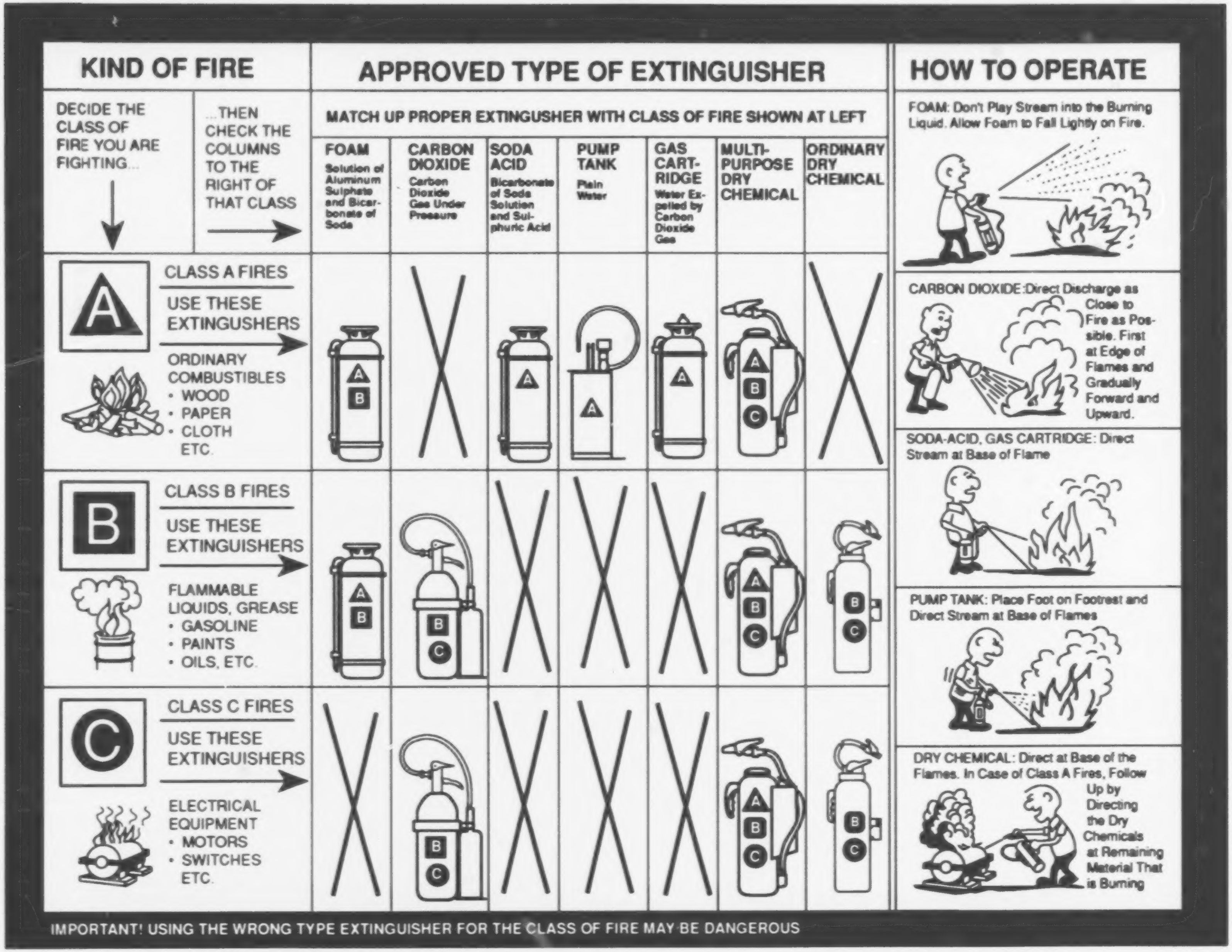
Extinguishers may contain foam, carbon dioxide, soda acid, water, dry chemicals or be a multipurpose type. Many institutions provide the multipurpose dry chemical extinguisher for use on any type of combustion. The symbols for the three types of fires have different shapes and are color-coded. The symbol for Class A fires is a green triangle; Class B is a red square; and Class C is a blue circle. These symbols will appear on the side of the extinguisher. Learn to recognize these symbols and remember what type of fire they indicate in order to select the correct extinguisher.

Flammable liquids require special care. They must be stored in a designated area, preferably in a fire safety cabinet labeled "FLAMMABLE—KEEP FIRE AWAY." To dispose of empty containers of flammable chemicals, first fill to overflowing with tap water. Repeat three times. (This is a requirement of the Environmental Protection Agency.) Then drain the containers, and place them in the disposal area as designated by hospital policy.

Routines to Follow in Emergencies

If a fire occurs, the laboratory must alert all personnel. Each worker must know his or her exact responsibility, how to report a fire, and the exit routes from the laboratory. All of these things are included in fire drills, which are held at regular intervals in most medical facilities.

Some institutions sound chimes to indicate a specific location of a fire by the number of rings. Others use codes announced over the loud speaker system. *Codes* indicate that an emergency has occurred and what that emergency might be. These calls are designed to prevent frightening patients or visitors in emergencies. Other emergency situations besides fires are included in the system. Health care



KIND OF FIRE

DECIDE THE CLASS OF FIRE YOU ARE FIGHTING...

...THEN CHECK THE COLUMNS TO THE RIGHT OF THAT CLASS

APPROVED TYPE OF EXTINGUISHER

MATCH UP PROPER EXTINGUSHER WITH CLASS OF FIRE SHOWN AT LEFT

KIND OF FIRE	FOAM Solution of Aluminum Sulphate and Bicarbonate of Soda	CARBON DIOXIDE Carbon Dioxide Gas Under Pressure	SODA ACID Bicarbonate of Soda Solution and Sulphuric Acid	PUMP TANK Plain Water	GAS CARTRIDGE Water Expelled by Carbon Dioxide Gas	MULTI-PURPOSE DRY CHEMICAL	ORDINARY DRY CHEMICAL
A CLASS A FIRES USE THESE EXTINGUSHERS ORDINARY COMBUSTIBLES • WOOD • PAPER • CLOTH ETC.	A B	✗	A	A	A	A B C	✗
B CLASS B FIRES USE THESE EXTINGUISHERS FLAMMABLE LIQUIDS, GREASE • GASOLINE • PAINTS • OILS, ETC.	A B	B C	✗	✗	✗	A B C	B C
C CLASS C FIRES USE THESE EXTINGUISHERS ELECTRICAL EQUIPMENT • MOTORS • SWITCHES ETC.	✗	B C	✗	✗	✗	A B C	B C

HOW TO OPERATE

FOAM: Don't Play Stream into the Burning Liquid. Allow Foam to Fall Lightly on Fire.

CARBON DIOXIDE: Direct Discharge as Close to Fire as Possible. First at Edge of Flames and Gradually Forward and Upward.

SODA-ACID, GAS CARTRIDGE: Direct Stream at Base of Flame

PUMP TANK: Place Foot on Footrest and Direct Stream at Base of Flames

DRY CHEMICAL: Direct at Base of the Flames. In Case of Class A Fires, Follow Up by Directing the Dry Chemicals at Remaining Material That is Burning

IMPORTANT! USING THE WRONG TYPE EXTINGUISHER FOR THE CLASS OF FIRE MAY BE DANGEROUS

Fig. 1. Fire safety

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institutions may devise a code for local use or use the International Hospital Code System. There are four codes in this system:

1. **Code Red** means that there is a fire in the hospital.
2. **Code Blue** means that someone has stopped breathing, or the heart has stopped beating, or both.
3. **Code Yellow** means that there is an uncontrolled individual or a threatening situation in the facility.
4. **Code Green** means that a special team of people should report to the emergency area. This code would be called when a disaster occurred.

A *disaster* is a sudden event that causes great damage or loss, such as a tornado, train wreck, or airplane crash. To prepare for such an event, hospital personnel participate in disaster drills with patients having simulated injuries. Students observing or working in a health care facility must know the meaning of the codes and how to conduct themselves according to local policy.

Disposal of Contaminated Materials

A serious problem in the laboratory is the safe handling of infectious materials. The Universal Precautions assume that all samples are potentially infectious. All specimens which *could* be contagious are considered *to be* contagious. All biologically contaminated trash is considered to be infectious. This includes laboratory materials such as sponges, glassware, syringes, and needles, as well as specimens from patients. There is no distinction between the disposal of samples known to be contaminated with HIV or hepatitis B and other samples. All samples are autoclaved or incinerated. Discarded specimens should be disinfected with sodium hypochlorite (bleach), then autoclaved. Finally, the entire specimen is placed in a heavy plastic bag showing the biohazard symbol and labeled CONTAMINATED and then incinerated. Large equipment that cannot be autoclaved may be soaked in bleach.

In case of spills of contaminated matter (blood, body fluids, or laboratory solutions), cover the entire area with paper towels. Then, flood with bleach. Next, wearing gloves, place soaked towels and spillage in a container for autoclaving. Flood again with bleach. Finally, clean as usual.

Other contaminated specimens are returned to the original container, placed in bags labeled CONTAMINATED, BIOHAZARD, and incinerated. Disposable laboratory equipment is similarly treated. Reusable equipment is soaked in disinfectant, then autoclaved.

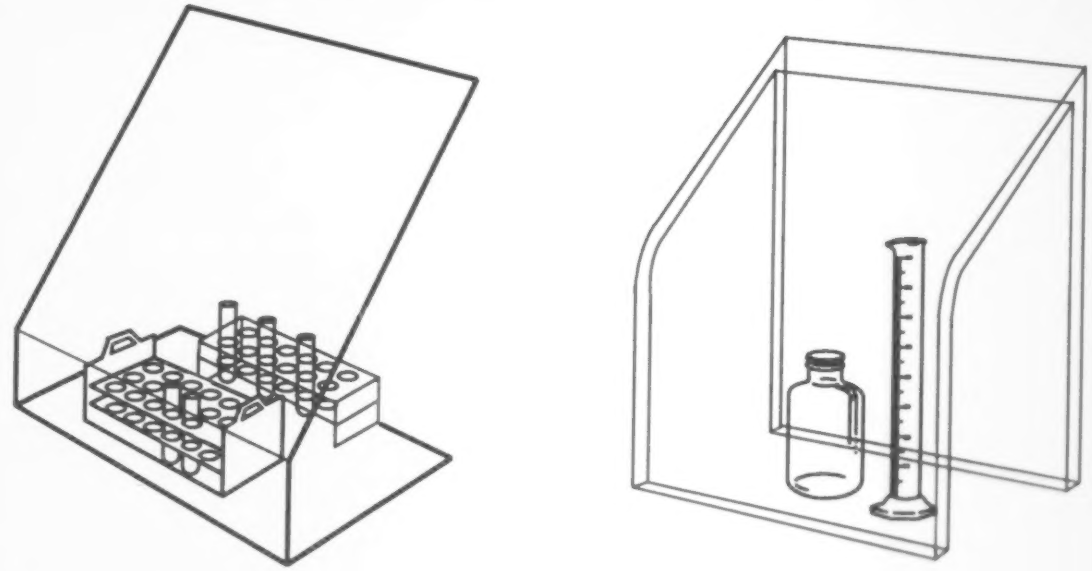
All sharps, needles, scalpel blades, and disposable syringes with needles attached should be placed in puncture-proof containers located in the area where they are used. Used needles should not be broken, bent, recapped, or handled with the hands but placed directly into the container.

Fluorescent orange or orange-red labels bearing the biohazard symbol must be placed on anything containing potentially infectious materials, such as refrigerators, freezers, medical waste receptacles, and shipping containers. Marked bags may be used instead of labels in some instances.

Equipment and Safety

Laboratory equipment in itself may be dangerous. Glassware can be particularly hazardous. Never use broken or chipped glass equipment. The time lost due to cuts from such equipment is more costly than replacement. If glassware is broken, clean up every particle. Tongs or forceps are used to discard pieces in a sharps container.

Any time there is a risk of splashing or aerosol formation when a worker opens a blood collection tube, some type of barrier should be placed between the worker and the specimen. Generally, clear transparent plastic screens are



Shield with rack of test tubes

Shield with cylinder and bottle

Fig. 2. Barriers or shields used for worker protection

used. These come in various sizes, thicknesses, and shapes. The screens also serve to protect against unexpected chemical reactions and injury from instrumentation or breakage of equipment. Figure 2 shows two type of barriers or shields.

A *safety shower* is part of the safety equipment in all laboratories. It should be centrally located and easily accessible to all workers. It is used to flush corrosives from the skin and clothing. Pulling the handle causes a large amount of water to flood the victim immediately.

An *eyewash station* is a small sink designed to direct water into the eyes. It is usually located close to the area where strong chemicals are used. If you should splash any toxic substance into your eyes, go at once to the eyewash station, and flood your eyes with water. Continue to flush the eyes with water for 15 minutes, or until medical treatment is begun. Keep your eyes open while flooding. The first 15 seconds after an accident to the eyes are crucial to the preservation of sight.

Ventilation hoods can be used to carry away fumes or to trap and contain contaminants. Hoods come in varying sizes and are most often found in the chemistry or microbiology sections of the medical laboratory.

Safety Practices in the Laboratory

There are many general safety practices that should be followed:

1. Always clean spills from the work area and floor immediately.
2. Report broken or hazardous equipment to the supervisor.
3. Never leave storage drawers open or cabinet doors ajar.
4. Always use an automatic pipette or a rubber bulb and pipette to transfer materials from one container to another. Never pipette by mouth.
5. When collecting specimens, be sure to check the patient's identification.
6. Do not use frayed or damaged electrical cords.
7. All reagent bottles must be labeled, and the labels must be legible.
8. Always read labels three times—as you reach for the bottle, as you pour from the bottle, and as you replace it.
9. Do not keep food of any kind, such as lunches or cold drinks, in the laboratory refrigerator.
10. No eating, drinking, or smoking is allowed in the laboratory.
11. Remove contact lenses while working with chemicals.

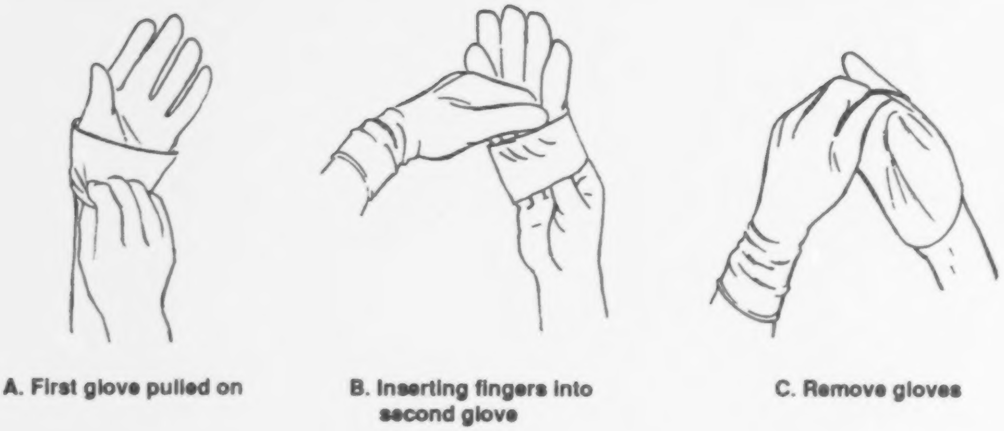

A. First glove pulled on
B. Inserting fingers into second glove
C. Remove gloves

Fig. 3. Donning and removing gloves

Protective Clothing

Medical laboratory workers deal routinely with all types of body fluids. Workers must protect themselves through handwashing and the wearing of protective apparel. Protective apparel consists of gloves, masks, eyewear, gowns, jackets, and aprons.

All workers are expected to wear gloves when doing something that could contaminate the hands. Gloves are not worn when talking on the telephone or using the computer. Once your hands have been washed, you are ready to glove. Take a disposable vinyl glove, and slip your fingers into it. Place a second glove on the other hand. Work your fingers into the interdigital spaces so that the gloves fit comfortably on each hand. When you remove them, catch the edge, turn them wrong side out, and discard them. Figure 3 shows the procedure for donning and removing gloves. Remember, when you are wearing gloves, you contaminate what you touch with your gloves, such as the telephone.

For some procedures, you may need to wear a mask. Place the mask over your nose and mouth, and pull the elastic over your head to a comfortable position. Discard the mask in the proper container when the procedure is complete.

Safety standards require the use of protective eyewear for some procedures. Eyewear may consist of special big-lens glasses or safety goggles. Face shields which completely protect the face and allow a person to wear eyeglasses underneath may also be used.

Wear a laboratory jacket or coat when working in the laboratory. Remove it when you leave. OSHA mandates that protective clothing be removed before leaving the workplace. Follow this procedure: clean and disinfect the work area, remove gloves, remove the laboratory coat or jacket, and wash your hands. The procedure for aseptic hand washing is shown in figure 4.

Exposure Control

Employers are required to offer hepatitis B vaccine to any worker who may be exposed to infectious materials at the employer's expense. The employer evaluates the potential for exposure to hazardous materials and communicates the information to the employee. A worker may refuse the vaccine by signing a waiver, but may later change his or her mind and receive the vaccine.

All laboratory workers are offered the vaccine. If the vaccine is refused, the employer could refuse to pay for treatment if hepatitis B is contracted. Also, proof of immune status for measles, mumps, and rubella is required at some facilities. If status is unknown, the immunization is offered. If it is refused and an employee is exposed to measles, mumps or rubella on the job, the employee is required to stay home until the incubation period is passed, usually without compensation.

Reporting Accidents

In spite of all efforts to practice safety, accidents may occur. If so, you must report the accident to your supervisor at once. The victim must be given proper first aid. Prompt corrective action must be taken if needed.

In a hospital, when an unusual event or accident has occurred outside routine procedure, it is called an *incident*, and an *incident report* must be filed with the administration. It must include (1) a description of the accident; (2) the time, place, and cause of the accident; and (3) names of witnesses. The incident report may be used for claims for workers' compensation or possible liability suits.

National Fire Protection Association's Symbols

The National Fire Protection Association (NFPA) has devised a series of emblems that indicate the presence of hazardous materials at a glance. All facilities that must have hazardous materials are advised to put this emblem on the outer door. This makes it easier for emergency personnel to determine problems quickly.

The symbol is a diamond divided into four equal parts, or smaller diamonds. Each small diamond is a different color. Blue indicates health hazard, red is for flammability, and yellow indicates reactivity (stability). These are always arranged in the same pattern (see fig. 5). Health hazard (blue) is indicated in the left diamond, flammability (red) at the top, and reactivity (yellow) at the right. The bottom diamond is left blank. It is to be used to identify other hazards, such as "biohazard," "radioactive," "do not use water," "flammable solid," "corrosive," and "sulfuric acid."

Summary

Two organizations responsible for enforcing safety regulations in the laboratory setting are OSHA and the Centers for Disease Control, Atlanta, Georgia. Fire safety, equipment, codes, plans, and procedures are all part of worker safety. Proper disposal of sharps and all types of contaminated specimens is very important. The procedure and purpose of making an incident report are given in the unit. The NFPA symbols for identifying hazards are displayed in figure 5 and discussed. Figure 6 has CDC precautions for health care workers in the laboratory.

Step 1: Don't let uniform touch sink. Use paper towel to turn on *warm* water.

Step 2: Wet hands, holding hands lower than wrists, and apply soap. Use liquid soap, if possible.

Step 3: Use strong rubbing movements to loosen bacteria from hands. Wash between fingers and thumbs.

Step 4: Rinse well, with hands lower than wrists. Inspect hands. If necessary, repeat the washing. Dry hands and arms well, and use paper towel to turn off water.

Fig. 4. Steps in washing the hands to prevent the spread of bacteria.

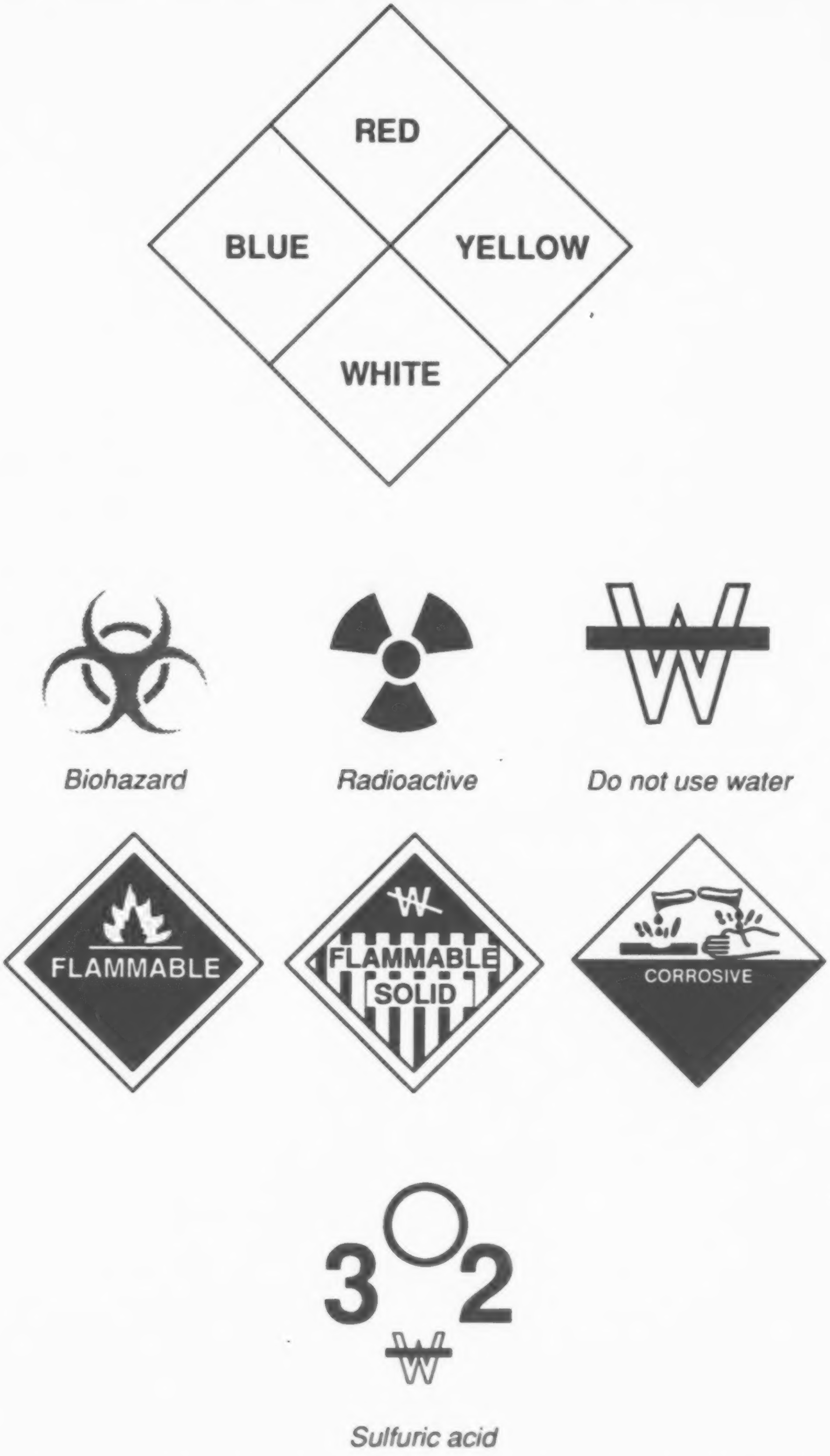

RED
BLUE
YELLOW
WHITE
Biohazard
Radioactive
Do not use water
FLAMMABLE
FLAMMABLE
SOLID
CORROSIVE
3
2
Sulfuric acid

Fig. 5. NFPA Emblem and Hazard Identification symbols. (Reprinted by permission from NFPA 49-1975, Hazardous Chemical Data. Copyright © 1975, National Fire Protection Association, Boston, Mass.)

Definition of Health Care Worker

Health care workers are defined as persons, including students and trainees, whose activities involve contact with patients or with blood or other body fluids from patients in a health care setting.

Precautions for Laboratories

Blood and other body fluids from **all** patients should be considered infective. To supplement the universal blood and body-fluid precautions, the following precautions are recommended for health care workers in clinical laboratories.

1. All specimens of blood and body fluids should be put in a well-constructed container with a secure lid to prevent leaking during transport. Care should be taken when collecting each specimen to avoid contaminating the outside of the container and of the laboratory form accompanying the specimen.
2. All persons processing blood and body-fluid specimens (e.g., removing tops from vacuum tubes) should wear gloves. Masks and protective eyeware should be worn if mucous-membrane contact with blood or body fluids is anticipated. Gloves should be changed and hands washed after completion of specimen processing.
3. For routine procedures, such as histologic and pathologic studies or microbiologic culturing, a biological safety cabinet is not necessary. However, biological safety cabinets (Class I or II) should be used whenever procedures are conducted that have a high potential for generating droplets. These include activities such as blending, sonicating, and vigorous mixing.
4. Mechanical pipetting devices should be used for manipulating all liquids in the laboratory. Mouth pipetting must not be done.
5. Use of needles and syringes should be limited to situations in which there is no alternative, and the recommendations for preventing injuries with needles outlined under universal precautions should be followed.
6. Laboratory work surfaces should be decontaminated with an appropriate chemical germicide after a spill of blood or other body fluids and when work activities are completed.
7. Contaminated materials used in laboratory tests should be decontaminated before reprocessing or be placed in bags and disposed of in accordance with institutional policies for disposal of infective waste.
8. Scientific equipment that has been contaminated with blood or other body fluids should be decontaminated and cleaned before being repaired in the laboratory or transported to the manufacturer.
9. All persons should wash their hands after completing laboratory activities and should remove protective clothing before leaving the laboratory.

Implementation of universal blood and body-fluid precautions for all patients eliminates the need for warning labels on specimens since blood and other body fluids from all patients should be considered infective.

Fig. 6. Precautions for health care workers in the laboratory. (The section, "Precautions for Laboratories," occurs on pp. A6-49, A6-54, and A6-55 of the Morbidity and Mortality Weekly Report Supplement, August 21, 1987, vol. 30, no. 25, published by the U.S. Department of Health and Human Services, Public Health Services, Centers for Disease Control, Atlanta, Georgia 30333.)

2

Organization of the Laboratory

Objectives: After completing this unit, you will be able to—

1. *identify titles, education, and functions of personnel working in medical laboratories;*
2. *explain how a large laboratory is organized;*
3. *distinguish work areas in the laboratory by name and function; and*
4. *describe uses of the computer in a health care facility and its application in the medical laboratory.*

Terms and Definitions

associate degree—awarded by a community or junior college after completion of a prescribed two-year course of study; called an associate degree (A.D.).

baccalaureate degree—awarded by a college or university after completion of undergraduate studies, usually for four years; called a bachelor's degree; e.g., Bachelor of Science (B.S.).

certification—issuance of a certificate by a regulating professional organization that a person has fulfilled standards of education and performance.

computer—electronic device with the ability to develop, store, and transfer data.

doctorate—highest degree awarded by universities; called a doctor's degree; e.g., Doctor of Philosophy (Ph.D.).

internist—specialist in internal medicine who treats illnesses of adults nonsurgically; sometimes heads laboratory where no pathologist is available.

laboratorian—laboratory worker.

licensure—permission to practice awarded by a state or national board to persons who have proved competency for an occupation.

master's degree—awarded by a college or university after completion of two to three years of additional study beyond a bachelor's degree; e.g., Master of Science (M.S.).

pathologist—examines cells and tissues to aid in diagnosis and treatment; heads laboratory; performs autopsies.

registry—list of qualified individuals kept on an official record, indicating satisfactory completion of standards for some profession.

terminal—work station for a computer consisting of a video screen and a keyboard.

The largest number of health careers is found in hospitals. These facilities employ about two-thirds of the health care workers. Other smaller facilities include clinics, nursing homes, and the offices of doctors, dentists, and veterinarians. Most of these facilities establish some type of laboratory. These vary in size from multidepartmental laboratories in large hospitals to the small one-technician laboratory in a doctor's office.

Laboratory Personnel

There are different levels of training for workers in health care facilities. Personnel in laboratories also have specific titles. In the medical laboratory, the *medical technologist*

(M.T.) is highly trained to carry out complex laboratory procedures. In some laboratories, he or she also supervises the work of other laboratory personnel. Several educational approaches are used to qualify as an M.T. The person may have a bachelor's degree and train for one year at an certified hospital, have three years of college plus one year of hospital training, or two years of college plus two years at a training hospital. The two latter plans lead to a bachelor's degree in medical technology. With the first plan, the medical technologist's degree may also be in a related field, such as biology or chemistry.

After training, the person must then successfully pass an examination. These examinations are administered by certifying organizations such as the American Society of Clinical Pathologists (ASCP), the American Medical Technologists (AMT), National Certification Agency for Medical Laboratory Personnel (NCAMLP), and the International Society for Clinical Laboratory Technology (ISCLT).

A *medical laboratory technician* (M.L.T.) is an individual who has completed two years of medical laboratory training in a community college and has passed a national accreditation examination. M.L.T.s perform basic laboratory procedures and routine tests.

Laboratory aides are trained on the job for several weeks. Their duties vary, but, in general, they clean equipment, glassware, and instruments; sterilize materials; and prepare specimens. They learn to be more and more useful as they assist the medical technologist. Many laboratory aides continue their education to qualify for more advanced positions.

A *phlebotomist* is someone who draws blood samples. They are trained on-the-job.

The *cytotechnologist* (C.T.) prepares and studies slides of human cells. The C.T. makes preliminary reports of abnormalities or evidence of disease to the pathologist to indicate further study is needed. The required training is two years of college and another year in a laboratory school. Accreditation is voluntary and available through registries such as the American Society of Clinical Pathologists.

Tissue samples are prepared for microscopic examination by a pathologist through the efforts of the *histotechnologist* (H.T.L.) or the *histologic technician* (H.T.). These pathology laboratory workers prepare and preserve body tissue for sectioning and staining before placement on slides. The histotechnologist has studied for four years in college plus one year in a laboratory school. The histologic technician has worked and studied for two years in a pathology laboratory or for one year in an accredited program. Certification is offered through the American Society of Clinical Pathologists.

Laboratory personnel who wish to continue their education may receive specialty certification in areas such as microbiology, hematology, clinical chemistry, virology, and other related areas.

Physicians

A *physician* diagnoses and treats patients and works to prevent disease and health problems. The training to become a Doctor of Medicine (M.D.) is long and difficult. It requires three to four years of college and then four more years of study in a medical school. An M.D. degree is conferred on graduation. The new M.D. must successfully pass a qualifying examination given by the State Board of Medical Examiners. After this, most states require that the M.D. work one year as an intern before beginning to practice medicine without supervision and consultation. If the doctor wishes to specialize in a particular branch of medicine, he or she will serve a *residency* by spending an additional two to five years working in a hospital under supervision to learn that specialty.

A *pathologist* is a medical specialist who examines body cells and tissues removed during surgery or at autopsy for abnormal conditions. A pathologist usually directs medical laboratory activities and consults with physicians about the results of tests. Small laboratories in clinics, lacking a pathologist, are sometimes headed by an internist.

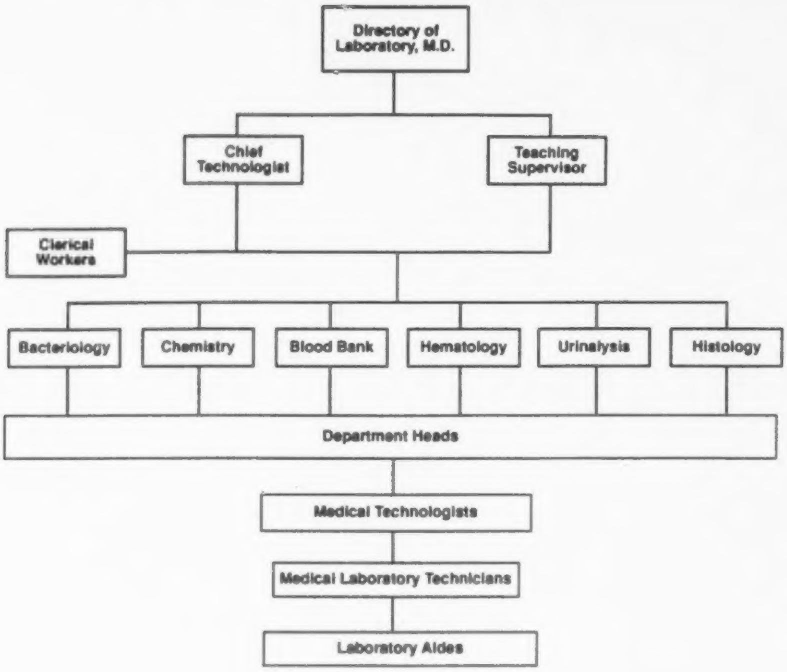

Directory of Laboratory, M.D.
Chief Technologist
Teaching Supervisor
Clerical Workers
Bacteriology
Chemistry
Blood Bank
Hematology
Urinalysis
Histology
Department Heads
Medical Technologists
Medical Laboratory Technicians
Laboratory Aides

Fig. 1. Organization of a large laboratory

Figure 1 shows the organization of a large laboratory. The chief technologist works closely with the pathologist, supervises all personnel and testing, and institutes new procedures as needed.

Work Areas Within the Laboratory

In large laboratories, a work area may be a large room or a department with several rooms. In a small clinic laboratory, the work area may be limited to a counter or portion of a counter. Not all work areas are represented in every laboratory. The names of areas are based on the types of tests performed or the function of the location. Major divisions of the laboratory include bacteriology, blood bank, chemistry, cytology and histology, hematology, microbiology, parasitology, pathology, serology, urinalysis, and a section for record keeping. Some of these sections may be combined or called by different names.

Besides growing bacteria in cultures, workers in *bacteriology* stain slides and use special culture media to identify specific types of bacteria. Sensitivity tests are run to determine the medication of choice and the proper dosage of the drug.

Donors are screened and their blood drawn in the *blood bank*. The units of blood are typed, carefully tested for purity, and placed in the bank for future use. Blood will be divided into its components for distribution to more than one person. When a patient needs blood, workers cross match units of blood to determine compatibility with the recipient's blood. Any reaction to a transfusion is investigated. Unusual types of antibodies are identified.

Laboratorians in *chemistry* analyze the contents of blood, urine, gastric juice, cerebrospinal fluid, and any body fluid to determine whether it is abnormal and how much it deviates from the normal.

Cytology and histology are often together. *Cytology* is concerned with cells discarded by the body while *histology* deals with tissues. Both accept tissues and cells for fixing, sectioning, staining, and mounting on slides. Technicians identify normal and abnormal cell structures, including cancer, during microscopic examination. Pathology usually refers to these sections of the laboratory. The other departments in the laboratory are called "the clinical laboratory" or "the medical laboratory." A morgue in a hospital may be closely associated with the pathology department since the pathologist is directly involved in all three areas.

In the *hematology* department, blood cells are examined as to number, morphology, type, and proportion to fluid. Abnormal cells are noted. Presence of infection and other diseases is detected. Cerebrospinal fluid is also examined. Studies on the coagulation ability of the blood are carried out in hematology.

Microscopic organisms, which cause disease, are isolated and identified in the *microbiology* department. These may be fungi, rickettsiae, and other parasites, as well as bacteria. Because of this variety, the term *bacteriology* has been changed to *microbiology department* in most laboratories.

Blood and fecal samples are studied in *parasitology* to test for the presence of parasites. Tests for the presence of blood are run on feces.

Phlebotomy may be a section in the blood bank where donor blood is drawn, or it may refer to a section of the laboratory where blood samples are collected for testing.

Workers in *serology* use antigens to test for the presence of antibodies in tissue or blood for diagnosis of a specific disease, including venereal disease. Pregnancy testing may be done here or in connection with urinalysis.

Urine is examined in *urinalysis* for physical properties, tested for chemical content, and examined microscopically.

One essential section of the laboratory is the bookkeeping or clerical department. Requests for tests and reports on the results of testing are collected and recorded here. Office supplies are furnished through this section.

Communication by Computer

Computers are electronic devices with the ability to create, process, and store data. This data can be retrieved as needed. Computers come in different sizes and have various uses in the health field. Extensive systems are found in hospitals, nursing homes, and clinics. With the development of small, less expensive models, computers have appeared in the private offices of physicians, dentists, chiropractors, and veterinarians. An office may have its own system or a terminal for input into a large processing service at a distant site. Linkage is by telephone. A *terminal* is the work station for a computer consisting of a video screen and a keyboard. A printer may be attached if printed reports or hard copy is needed. A work station is shown in figure 2.

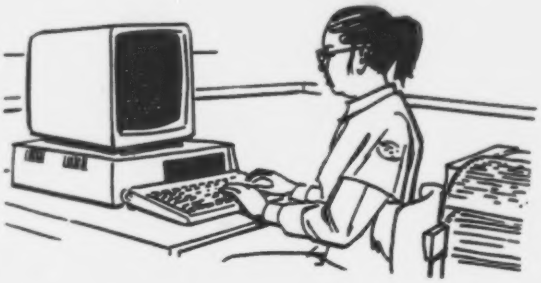


Fig. 2. Work station with terminal and printer

The two major divisions of a medical information system are patient care and business management. Under these divisions are programs to deal with bookkeeping, billing, correspondence, supply inventory and management, patient data, and clinical support. A computer system provides speed, accuracy, and efficiency not possible with manual record entry.

In a hospital, a computer terminal is placed at each nursing station and in each department. The screens should be placed to ensure confidentiality of what appears on the display. Doctor's orders can be put into the computer and transferred to the laboratory to order tests. Results can be reported by computer printout for inclusion in the patient chart. An entry is made as the service is provided, together with the cost, and transferred to the business office for billing.

In the record room, a patient's chart is incorporated into a data bank. The present admission together with records of past admissions is stored here. This information is available to authorized personnel in the laboratory.

Within the laboratory, computers have many uses besides receiving and dispensing patient data. Other uses are preparing labels for identification of patient and tests, running the automated equipment to test patient samples, evaluating quality control procedures, and preparing reports of test results. Computers can be used to order supplies and to update inventories.

Summary

The titles, educational requirements, and functions of personnel working in the medical laboratory are listed and reviewed. Figure 1 shows the organization of a large laboratory. Laboratory work areas are described. Computers are found in hospitals and doctors' offices. These are electronic devices that process, store, and retrieve data. The quality of patient care and business management is improved and made more efficient through the use of data systems.

3

Collecting and Processing Specimens

Objectives: After completing this unit, you will be able to—

1. *receive and dismiss a patient;*
2. *put patients of all ages at ease during collection of specimens;*
3. *explain how to protect patient information ethically;*
4. *differentiate between venipuncture and capillary blood collection;*
5. *discuss methods for collecting fecal and urine specimens;*
6. *describe the types of specimens received in a laboratory;*
7. *specify how different types of specimens are processed; and*
8. *explain how specimens are labeled and logged into records.*

Terms and Definitions

anticoagulant—substance that prevents blood from clotting (coagulation).
apprehensive—anxious, worried.
bevel—the slanted tip of a needle.
biopsy—to take out a small piece of tissue for microscopic examination.
blood plasma—the liquid part of the blood that has been prevented from coagulating.
blood serum—the clear liquid part of the blood that remains when a clot forms.
catheter—a tube put in the body to permit injection or withdrawal of fluids.
coagulation—clotting of blood.
hemolysis—rupture of red blood cells with the release of hemoglobin into the plasma or serum.
hostile—unfriendly; acting like an enemy.
lancet—a sharp-pointed, two-edged surgical knife.
lipemia—abnormal amount of fat appearing in the blood.
rapport—a relation marked by harmony.
resection—to cut off a portion of bone or other body structure.
supernatant—the clear liquid remaining at the top after the heavier parts settle to the bottom.
venipuncture—puncture of a vein, usually for withdrawing blood.

Whether you work or observe in a small clinic or in a large hospital laboratory, you will receive patients. You may be the first professional person they see. It is your responsibility to make them welcome and to try to establish a comfortable relationship. Every patient, regardless of social or economic status, has a right to expect courteous treatment no matter how busy you may be. All patients' problems are of paramount importance to them, even if they may seem routine or trivial to you. Remember, no one wants to be ill. An attitude of friendliness, concern, and optimism helps patients to cope with their problems. Moreover, you will get great satisfaction from helping patients.

Developing Good Patient Relationships

Rapport is a relation marked by harmony. The following suggestions could help to establish a good relationship leading to rapport with the patient.

Use the patient's name. This is important for identification and for indicating interest in the patient as a person. Make a real effort to pronounce each name correctly.

Treat every patient as an important person. Make the patient aware that you are there to serve. Listen to what the person has to say. A friendly comment will almost always bring about a friendly response.

Explain the procedure the doctor has ordered. Mention any way in which the patient may assist in the procedure.

Try to reassure patients if they talk about their fears. Laboratory screening may be the first step of treatment for a frightened person facing a very real problem. Listen carefully, and show genuine interest. Some patients can admit fears to you that they feel they must hide from family and friends. Offer sympathy. Never joke or laugh about such fears.

If a patient is hostile, respond calmly. Hostility is often the expression of fear or worry. It may indicate that the patient is frightened, lonely, feeling helpless, or has the pressure of problems at home or at work added to the anxiety of illness. Remain calm and reasonable. If a patient refuses a test or procedure, do not argue. Simply report the refusal to the physician or supervisor in the laboratory.

Many people are frightened by the sight of needles and lancets. It is well to remember that such patients need extra assurance and consideration when blood is collected.

If there must be a delay, explain the reason to the patient. Estimate the length of extra time involved. Nothing adds to the fears of patients as much as waiting for appointments. Not only are they apprehensive, but a long wait implies that they are not important, that you are making them waste their time.

Special Considerations for Children

When teenagers and older children are approached for testing, they are treated as adults. Explain what you plan to do, and proceed. Young children and infants do not understand directions and cannot comprehend why they must be "hurt." Immobilize the child if necessary, and proceed as rapidly as possible. Sometimes parents may be asked to hold the child. At other times, a disinterested adult should restrain the infant, after the parent has left the room. Never tell a child "this isn't going to hurt."

Dismissing the Patient

When tests and procedures have been finished according to the doctors' orders, courteously instruct patients about the next procedure, or direct them to the next department. You may need to escort them to the next station because the medical facility may seem frightening and confusing to a patient.

Professionalism

People who work in medical laboratories are considered professionals. They are expected to conduct themselves ethically. One example of required ethical behavior is the privacy of the patient's record. The results of testing cannot be given to the patient, family, or friends. Patient information can only be discussed with the health care workers involved directly with the care of the patient.

It is sometimes hard to avoid discussing laboratory results when meeting with a patient for a second time or on a continuing basis. Nevertheless, it is always the responsibility of the doctor to give the patient test results. The laboratory worker never gives test results to the patient.

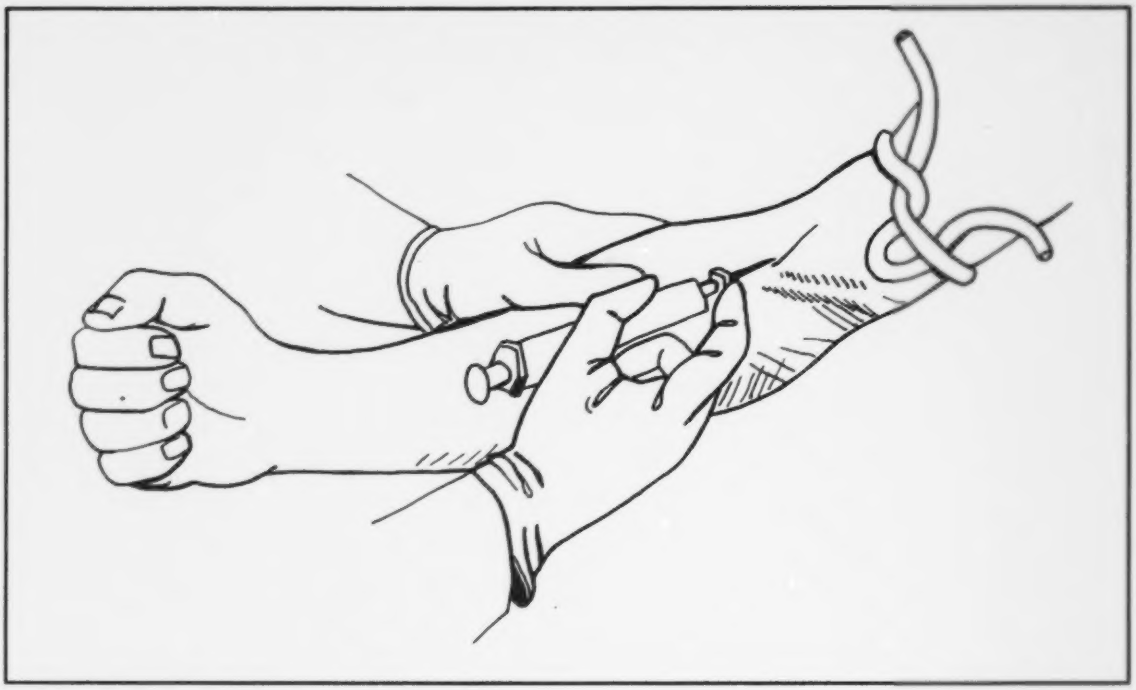


Fig. 1. Taking venous blood

Collection of Blood Samples by Venipuncture

Blood samples are used for many laboratory investigations. If more than a few drops are needed, blood is collected by venipuncture. It is important to establish rapport with the patient before you begin this procedure. Identify yourself, and tell the patient why you are there. Carefully identify the patient, using the name tag, arm band, and bed tag, and call the patient for his or her name. Review the doctor's orders to make sure you are collecting the correct amount of blood and that you have prepared the correct containers to receive the specimen.

You should appear calm, reassuring, and sympathetic to the patient. Remember that, although you may collect many blood specimens every day, the procedure is probably new and frightening to the patient.

Place the patient in a comfortable position. Ambulatory patients should be seated so there is a place to support the arm. Bed patients will remain in bed.

Briefly explain the procedure, but avoid going into detail about the tests to be performed. Your information may confuse the patient. Explain that there is little discomfort and no residual effect from venipuncture.

Venous blood is usually taken from veins in the inner bend of the elbow (fig. 1). If for some reason the elbow cannot be used, or if you have difficulty getting blood from these veins, other sites may be used. These include the lower arm, wrist, back of hand, fingers, lower leg, ankle, or upper surface of the foot. However, these veins are usually very small although they may appear large. They have a tendency to roll, and they may have toughened walls. Therefore, the veins in the elbow are almost always most satisfactory for venipuncture.

You will need to assemble your equipment before you collect blood. If you are using a syringe, you will need sponges or cotton balls, a tourniquet, a syringe with a needle, test tubes, and slides, if required.

Next, select the site for puncture. Place the tourniquet above the site, and tie it with a slipknot so it can be removed with one hand. Ask the patient to open and close the hand causing the veins to become congested. Then choose a vein that is clearly evident and seems so well supported by tissue that it will not roll.

Cleanse the area with a sponge and antiseptic such as alcohol or soap solution. Dry with a sterile sponge, or air-dry.

Insert the needle cleanly and rapidly with the bevel upward. Pull back on the plunger. As blood appears in the barrel of the syringe, you are assured that the needle is in the vein. Then release the tourniquet, and collect the amount of blood required.

Place a sponge over the puncture site, but do not apply pressure until the needle is withdrawn. Then apply pressure firmly, and flex the arm until bleeding stops. Caution the patient that a bruise will result if the wound is allowed to bleed.

Make blood smears if required. Then remove the needle from the syringe, and carefully distribute blood to the indicated containers. Do not force blood through the needle, nor handle roughly, because this may cause the red blood cells to rupture with resulting hemolysis. Label all tubes and slides. Check to be sure the patient is all right, then dismiss the patient or leave the room, taking all of your equipment with you.

If you are using a vacuum tube to collect blood, the procedure is similar. You will assemble sponges, a tourniquet, vacuum tube, and needle. Screw the needle into place on the adapter (holder). Vacuum tube collection equipment is shown in figure 2.

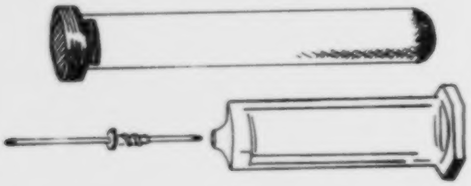


Fig. 2. Vacuum equipment for collecting blood

Place the tube loosely into the holder contacting the double-pointed needle, but not puncturing the stopper. Follow the venipuncture procedure to prepare the patient. Insert the needle into a vein.

After the needle end is correctly inserted into the vein, push the stoppered tube firmly onto the adapter point of the needle, puncturing the stopper. Continue to aspirate until the tube fills to the predetermined amount. Vacuum tubes will not fill completely.

If multiple samples are needed, remove the first tube, and rapidly replace it with a second, third, etc. Be careful to keep the needle in place inside the vein as you change tubes.

When all specimens have been collected, remove the needle, and care for the patient as described earlier. Label all tubes carefully. The label should include the patient's name and hospital number, the doctor's name, the date, and the test required. It may include the name of the collector, the patient's room number, and the time of collection, or any pertinent information.

Dismiss the patient. Always thank patients for their cooperation, and leave with a friendly attitude.

Tips on the Collection of Venous Blood

For some tests, uncoagulated blood is required. An *anticoagulant* is a substance that prevents clotting of blood. The most popular anticoagulants are oxalate (a mixture of ammonium and potassium oxalate), Sequestrene (EDTA), sodium citrate, and heparin. Be sure to select the correct one for the test to be done. The vacuum tubes are color-coded so that the stopper indicates which anticoagulant is in the tube. Blood placed in a test tube containing an anticoagulant must be inverted gently to mix. Do not shake the tube.

If serum is needed for a test, whole blood is collected in a plain tube. Do not invert or mix. Mixing may cause hemolysis of the serum. The blood should be allowed to clot for 30 minutes at room temperature.

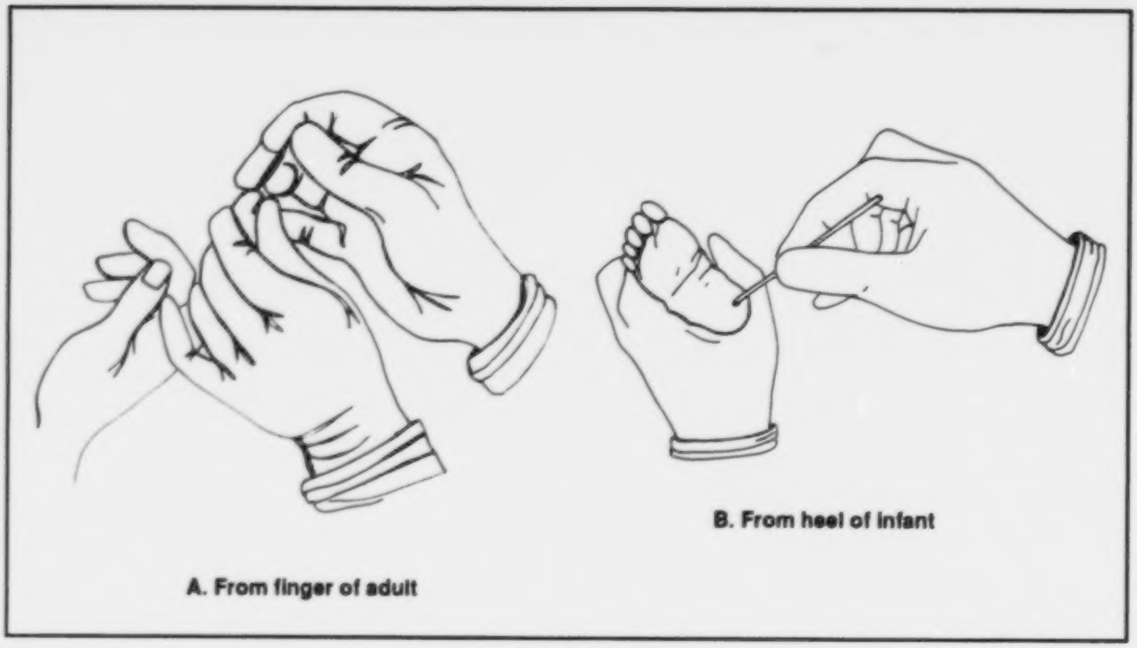

B. From heel of infant
A. From finger of adult

Fig. 3. Collecting capillary blood

Blood placed in a test tube containing thrombin or SST is inverted and can be centrifuged after 5 minutes.

The following tips will be useful in preventing hemolysis of blood during venipuncture:

- Use a sharp, smooth needle of appropriate size, usually 20 or 22 gauge.
- Enter the vein as directly as possible.
- Do not tie the tourniquet too tightly, and release it before aspirating blood.
- Remove the needle before distributing blood to tubes.
- If serum is needed, allow clot to form before centrifuging.

Collection of Capillary Blood

Capillary blood is collected when a few drops are enough for testing. Usually the specimen is collected from the ring finger or middle finger. The earlobe may be used if fingers are thick-skinned, very rough, swollen, scarred, bruised, or if the patient will be handling contaminated materials. The toe is used if neither a finger nor an earlobe is available. In infants, either the toe or the heel is used.

The first steps in collecting capillary blood are the same as those for venipuncture. Establish rapport with the patient. Identify yourself. Identify the patient by tag and by asking for his or her name. Review the doctor's orders.

Then assemble the equipment. You will need sponges, a sterile lancet, capillary tubes, blood pipettes, and diluting fluid or a diluting unit for the test needed, and slides.

Next, select the site to puncture. Cleanse the area with a disinfectant sponge, and wipe with a dry sponge. If the site is wet, the blood will spread out instead of forming a drop.

Grasp the finger firmly across the middle or first joint (fig. 3). The patient may involuntarily move if you do not hold the finger securely. Quickly puncture the skin with a sterile, disposable lancet. Wipe off the first drop of blood. To take advantage of gravity, keep the patient's hand conveniently low. Using gentle pressure on the finger, fill containers as ordered. Do not allow air bubbles to form in collection tubes or pipettes. Collect with a continuous flow of blood. Hold up the distal end of the collection tubes. Then seal the ends of capillary tubes with clay.

Finally, prepare blood smears on microscope slides if requested. Then cleanse the finger with a disinfectant sponge, and apply pressure with a dry sponge to stop bleeding.

Be sure the patient is all right. Thank the patient for cooperating, and then dismiss her or him. If the patient is in a hospital, be sure to collect all your equipment and the specimens before you leave the room.

Collection of Fecal Specimens

Laboratory tests on feces are important in diagnosis. The patient should be carefully instructed in the method of collection to ensure a satisfactory specimen. Hospital patients are given a clean, dry bedpan for defecation. Nursing personnel will collect the specimen and send it to the laboratory. If the patient is to collect the specimen, then he or she must fully understand what is to be collected, how it is to be collected, and what container should be used. The following general instructions will be helpful:

1. The specimen cannot be contaminated with urine.
2. No laxative should be taken unless it is ordered by the doctor.
3. A wide-mouthed container with a tight-fitting lid and a few wooden tongue blades or special collecting spatulas should be provided.
4. The patient may defecate in a clean, dry bedpan, a disposable paper collection unit, or on a paper plate.
5. The patient will collect a small amount of feces (size of a pecan), place it in the container, and cover securely. The patient must take care to avoid contaminating the outside of the container.
6. Finally, the patient must wash hands, attach completed label, and return the specimen to the laboratory.

The information on the label should include the patient's name, the time and date of collection, the name of the doctor, and any other pertinent information.

Fecal material collected by the doctor during rectal examination is sometimes sent to the laboratory for study.

A variety of tests may be performed on fecal specimens. Some of these require very specific instructions for collection. Details such as preservatives, special containers, exact time of collection, or a constant temperature may be important. Such instructions should be made very clear to the patient. Some samples of types of tests are these:

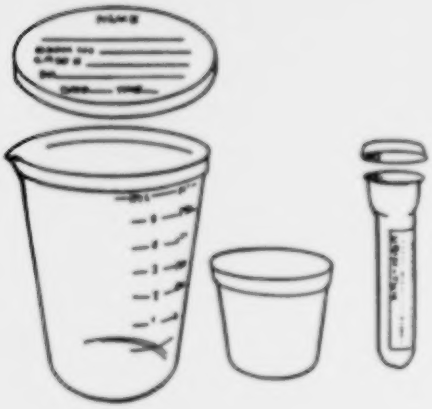
1. Gross examination for consistency, color, mucus, or blood
2. Occult blood
3. Detection of ova and parasites
4. Viral and bacterial cultures

Collection of Urine Specimens

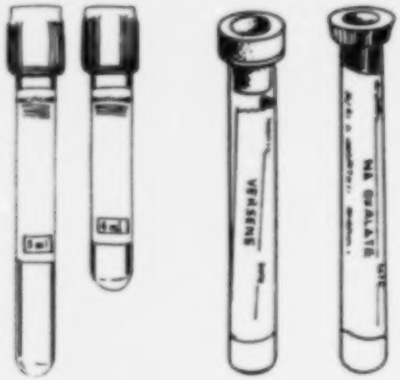
Perhaps the most common laboratory procedure is the urinalysis, which is a series of tests done on urine. A ***routine urinalysis*** means the series of urine tests selected by a given institution to be performed on most urine specimens. Usually, a routine urinalysis includes a gross examination of appearance, reaction, simple chemical tests, and a microscopic examination of sediment. A ***routine specimen*** means a urine specimen collected at any time during the 24-hour day. Such a specimen will vary in concentration depending on the patient's liquid intake. Sometimes the first urine specimen collected in the morning is preferred because it will be more concentrated. A routine urine specimen is almost always collected upon admission to the hospital or when visiting the doctor's office.

For some tests, a timed urine specimen, either 12 or 24 hours, is needed. This means that the entire quantity of urine voided for the timed period is collected and sent to the laboratory for testing.

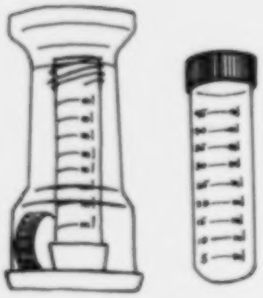
Most hospitals use plastic, disposable containers. Plastic, glass, or metal containers are commonly used in smaller laboratories because they may be washed, dried, and reused. Occasionally, a patient may be asked to collect a urine specimen at home. It is advisable to provide the patient with a specimen container. Be sure the container is clean, dry, and uncontaminated. Figure 4 shows specimen containers for urine.



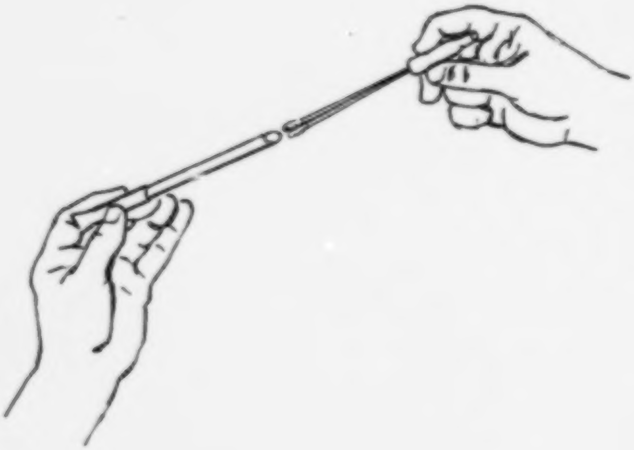
URINE: collected in plastic cup or plastic centrifuge tube.



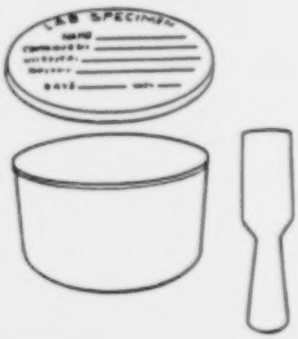
BLOOD: collected in tubes with stoppers, color-coded to indicate type of anticoagulant present in tube. (Two tubes on left are VACUTAINER PLUS tubes with HEMOGARD closure. Used with permission of Becton Dickinson VACUTAINER Systems)



SPUTUM: placed in plastic collection container. (Drawing of Sputum Collection System used with permission of Becton Dickinson VACUTAINER Systems)



SWAB: comes in a sealed sterile collection tube.



FECES: placed in disposable cardboard container.

Fig. 4. Specimen containers

Types of Specimens Received

Many types of specimens are received in the medical laboratory. The purpose of collecting specimen, is threefold: (1) to diagnose disease, (2) to determine treatment, and (3) to define progress of treatment. The amount needed depends on the tests to be performed. Specimens include any body excretion such as urine, feces, or sputum. Other specimens may be obtained by needle aspiration, such as blood, spinal fluid, pleural fluid, and body cells from the liver, sternum, or kidney. Tissue obtained from resections or from biopsies is sent to the laboratory for examination.

Another type of laboratory specimen is a *smear*. This is obtained by touching a body area with a cotton swab, then smearing the swab on a microscope slide. Such material is most commonly obtained from the nose, throat, or a wound. Many other types of specimens are received in the laboratory, depending on the capability of the laboratory and the need of the patient. Containers used to bring specimens to the laboratory for testing are shown in figure 4.

Logging Specimens

Most laboratories have an established routine for receiving specimens. An area is designated where specimens may be placed when they arrive in the laboratory. The medical laboratory worker must identify incoming specimens and route them to the correct work area.

Some method of *logging* or recording specimens must be used. The method depends on the type of institution, the type of specimen, and the number of specimens handled.

In very large laboratories, numbering systems and color coding are used. The color coding indicates the various divisions of the laboratory. This makes it easier to route specimens quickly to the correct division. Numbers may be used to identify the specimen, the accompanying information, and all the tests required to complete the laboratory examination. A permanent record of the test may be filed using the same number. There are many types of numbering devices on the market, such as numbered adhesive tapes or labels. Labels with bar codes are available to place on collection tubes and subsequent tests from that specimen. Bar codes are identified by computer (see fig. 5).

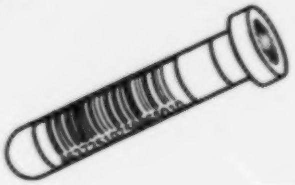


Fig. 5. Test tube with bar code label

In smaller laboratories, where there are fewer specimens to handle, simpler logging methods may be used. A request form with a single carbon copy may be satisfactory. This form indicates the type of test ordered and all the information needed to identify the patient, including the patient's chart number. This number is affixed to the specimen. When testing is complete, the original request, with test results, is returned to the patient's chart, using the number to ensure identification. The copy is filed in the laboratory.

In very small laboratories, specimens may be numbered with a marking pencil, and the same number recorded on the accompanying request sheet. Whatever method of logging is used, mistakes in identification of specimens are intolerable. All logging methods should be aimed at elimination of error.

Every means possible should be used to identify a specimen with incomplete information. Keep in mind that no laboratory specimens are unimportant. Some are extremely difficult to collect. Indeed, it is impossible to replace most laboratory specimens. If it is not possible to identify a specimen correctly, it must be discarded.

Preparing Specimens for Testing

The medical laboratory worker may be asked to process specimens. *Processing* is the preparation of specimens for actual analysis after they have been collected. One problem to avoid in processing blood is hemolysis.

Whole blood should be kept in the original stoppered container until it is ready for analysis. If there is a delay of more than an hour before testing, the specimen should be stored in the refrigerator.

Some blood tests are done using serum. ***Serum*** is the liquid part of blood that remains when a clot forms. To process a blood specimen, allow the blood to clot for 30 minutes. Then, rim the tube with an applicator to loosen the clot. Next, centrifuge the specimen, and collect the supernatant serum.

If an anticoagulant is added to whole blood, the liquid part of the blood in which the cells are suspended is the ***plasma***. To collect plasma, centrifuge the blood. The cells will then collect at the bottom of the tube. The supernatant fluid is plasma. Plasma should be refrigerated if there must be a delay between processing and analysis. Both serum and plasma may be frozen if the delay is longer than 4 hours.

Blood collected soon after a patient has eaten a meal may contain neutral fats. These will cause the serum or plasma to have a milky appearance (lipemia) and cause an error in test results. To avoid this problem, be sure to instruct the patient to fast before the blood is collected.

Other materials for testing, may require special processing, but the general rule is to examine the specimens as quickly as possible after collection. If prompt analysis is impossible, use the appropriate means to preserve the specimen.

Packaging Outgoing Specimens

Few laboratories are equipped to conduct every laboratory test that may be required. Some specimens must be sent to other laboratories. Outgoing specimens must be packaged in correct or suitable containers. They must be correctly labeled. The label should include all pertinent information. This includes the patient's name, address, and chart number; the date and hour of collection; the type of specimen; the exact tests ordered; and the name and address of the attending physician. This same information should be filed in the sending laboratory to ensure identification of the specimen, to verify having sent the specimen, and for patient billing. Special leakproof packaging is available for body fluid samples that must be sent by airmail for urgent testing.

Maintaining Files of Records or Reports

Copies of every laboratory test performed are kept in the medical facility, usually as a database in a computer. These records are used to determine charges for laboratory services, to replace reports that may have been lost, and to provide information and statistics for research.

Modern electronic equipment has made it possible to keep large numbers of laboratory records and reports in a small space. In some laboratories, however, medical laboratory workers may be required to file laboratory reports by hand.

Reports may be filed under the patient's name, patient's number, type of test, or even the name of the attending physician. Whatever method is used, filing and retrieval should be an easy task.

Summary

The relationship that should exist for rapport between patient and laboratory worker is presented. Collecting, processing, labeling, and logging specimens are discussed. The procedures for collecting specimens of venous blood, capillary blood, feces, and urine are outlined. The purposes of collecting specimens are listed. Types of samples are described. Methods of labeling specimens are described. Finally, methods for the filing of reports are given.

4

Laboratory Equipment

Objectives: After completing this unit, you will be able to—

1. *explain how equipment should be stored;*
2. *identify and use graduated cylinders, beakers, flasks, and test tubes;*
3. *identify and use volumetric and serological pipettes;*
4. *use a centrifuge and describe specialized types of centrifuges;*
5. *assist with calibration of tabletop centrifuges;*
6. *identify parts and uses of a microscope;*
7. *use an autoclave and an oven; and*
8. *use constant temperature appliances.*

Terms and Definitions

autoclave—apparatus for sterilization by steam under pressure.

calibration—mark on a piece of glassware or equipment that indicates the amount it will measure.

centrifuge—a machine that spins at high speed to separate materials of different densities.

graduated cylinder—straight-sided tube with a base for measuring the volume of liquids.

meniscus—a crescent-shaped curvature found on the upper surface of a liquid as it is measured.

pipette—a cylindrical glass tube used to measure and to transfer liquids; may be calibrated.

serological pipette—pipette that is graduated to measure various volumes within a given amount (e.g., 5 ml graduated in 0.10 ml).

sterilization—to make free of all living organisms.

thermostat—an automatic device that regulates or controls temperature.

volumetric pipette—a transfer pipette that measures a single amount.

Abbreviations

rpm—revolutions per minute; unit of measure for the speed of centrifuges.

TC—to contain; marked on pipettes.

TD—to deliver; marked on pipettes.

Every medical facility must store supplies and equipment. The methods used depend on the needs of the individual institution. All supplies should be kept where they are easily accessible, yet protected from damage.

Storage Areas

Most hospitals have a large storage area. Supplies are ordered in quantity and dispensed as needed. Usually, a written request is required. Some facilities have computer capabilities for ordering supplies. Computers also facilitate updating of inventory in the supply area.

In small offices, the medical laboratory worker may be assigned the task of maintaining supplies. A list of all equipment and supplies must be prepared. Regular inventory will tell what has been used and what must be ordered. A pad and pencil located in the supply area to record needs as they occur will make it easier to remember items needed. A list of suppliers with names and telephone numbers or sales representatives should be prepared and kept up to date.

An accurate file of operating manuals, warranties, and service personnel for all equipment should be prepared and maintained. One factor to consider in the purchase of equipment is the availability of maintenance service.

Some logical method of storage for glassware and disposable equipment must be devised for both efficiency in use and to avoid breakage. Glassware must be easy to find, whether it is stored by size, type, use, or a combination of all three. Pipettes and test tubes are usually stored in drawers built to hold specific sizes. Other glassware is stored on accessible shelves. Glassware that is not used often should be covered to keep dust out.

Large pieces of equipment should be stored out of the way. That which is most often used should be the most accessible. Labels may indicate not only the name of the equipment, but also what test it is used for and if a hazard is present.

Measuring with Calibrated and Volumetric Glassware

Graduated cylinders are designed to measure various quantities of liquid in graduated amounts. A *calibration* is a mark on the glassware that indicates the amount it will measure. For example, a 100-ml cylinder is graduated from 1 ml through 100 ml. Note the two sets of numbers on the cylinder. Begin reading the scale at the bottom of the cylinder. One set of numbers begins at 100 and goes upward to zero. The other set starts at zero and rises to 100. Thus, if 85 ml of liquid is poured into the cylinder, you will read the 85 ml on the side beginning at zero. Readings of volume of liquids must always be made at the *bottom* of the meniscus. A *meniscus* is the crescent-shaped curvature a liquid assumes when it is placed in a slender container. To read a volume of liquid in calibrated or volumetric glassware, the bottom of the meniscus is read against the graduation or calibration. The sighting should be done at eye level (fig. 1).

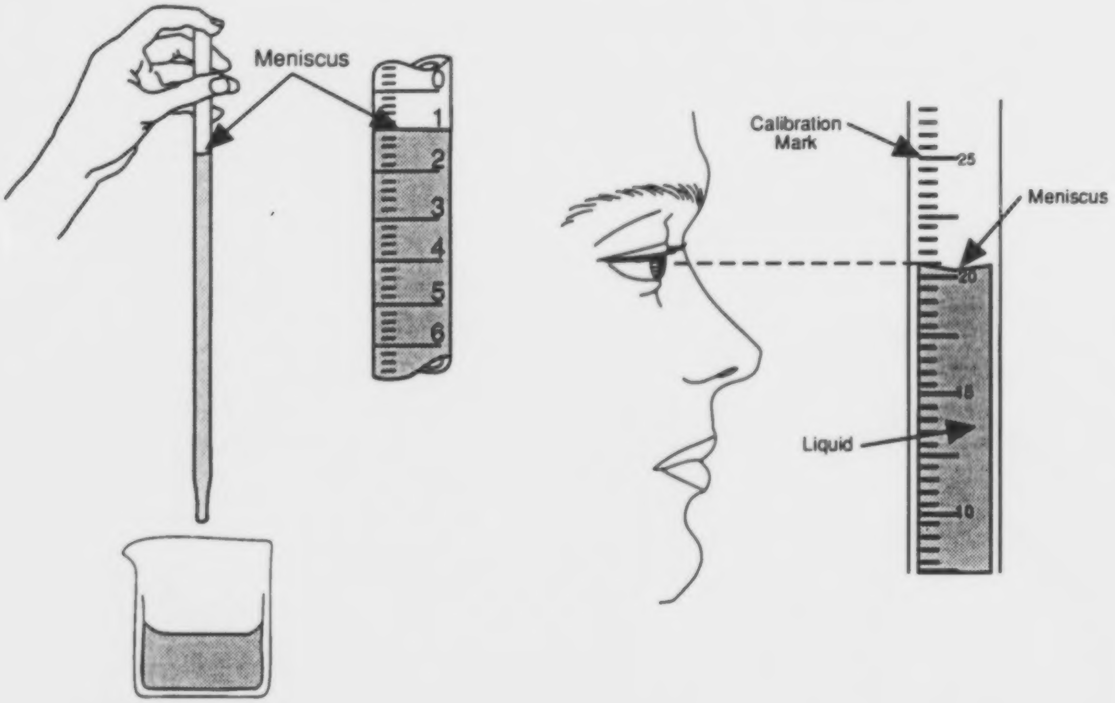


Fig. 1. Measuring with calibrated glassware

The flasks used most often in the laboratory are the volumetric, Erlenmeyer, and Florence flasks. Volumetric flasks are designed to contain a single given quantity. They range in size from 1 ml to 2,000 ml and have one calibration on the neck of the container. They usually have a ground glass stopper and are used primarily to prepare specific volumes of reagents. In using volumetric flasks, always read the bottom of the meniscus against the calibration mark.

Erlenmeyer flasks range in size from 50 ml to 2,000 ml volume. Calibrations extend up the sloping side from a flat bottom. The markings on the side can be used to measure or combine volumes not requiring absolute accuracy.

The Florence flask is similar to the Erlenmeyer except that the sides are rounded. It is available in the same volume capacities and can be used for purposes similar to the Erlenmeyer. Figure 2 shows examples of these flasks and other glassware.

A beaker is a straight-sided container. There is a pouring spout formed by the rim on one side. Beakers may show a volume capacity or have calibrations along the side. These containers are used for holding liquids or mixing when volume is not critical.

Test tubes come in a variety of sizes and shapes and have varied uses in almost all departments in a medical laboratory.

Using Pipettes

A *pipette* is a calibrated glass tubes open at both ends, used to measure small volumes of liquid. A pipette is also used to transfer liquids from one container to another. These are called transfer pipettes. A volumetric pipette or a medicine dropper is an example of a transfer pipet. Volumetrics are calibrated exactly. Dropping pipettes, which deliver drops, can be used when accuracy is not required. The two general types of calibrated pipettes are serological and volumetric (fig. 3).

Serological pipettes are graduated to measure various volumes within a given amount. These generally require filling to a calibration mark, then allowing to drain. They are etched at the mouth end and marked TD (to deliver). These pipettes must have the last drop blown out. A 10-ml serological pipette is calibrated in either 0.1 or 0.2 ml. The amount is stated on the pipette as "10 ml in 1/10" or "10 ml in 2/10." These pipettes can transfer a partial volume or their total volume.

Volumetric (transfer) pipettes, on the other hand, have only one calibration mark. This pipette is also a TD type. It should be allowed to drain down the side of the vessel. If a volumetric pipette is marked TC (to contain), allow the pipette to drain, then rinse with the diluting solution so the pipette will deliver the amount stated.

To use a pipette, attach a pipette bulb to the mouthpiece, place it into the solution to be measured, and use suction to draw the solution up to the desired mark. Be sure that the meniscus is on the desired calibration mark (see fig. 1). Withdraw the pipette. Wipe the outside with a tissue to dry it. Place the tip of the pipette against the side of the receiving vessel. Release the suction, and allow the liquid to drain into it. If the top of the pipette is etched, blow out the last drop. Use a mechanical suction device when pipetting concentrated acids and alkalis, deadly poisons, corrosive substances, and radioactive materials. Refer to figures 4 and 5.

Even smaller volumes of liquids can be transferred with micropipettes. These are automatic pipettes calibrated for less than 0.5 ml in volume. They are preset to draw up and dispense a small accurate volume. Some dispensers have multiple delivery tips and dispense reagents to a row of tests at one time. An automated analyzer has pipetting built into its system, thus eliminating this risk for the laboratory worker. Examples of these hand-held dispensers are shown in figure 6.

The Centrifuge

The centrifuge is one of the most commonly used pieces of equipment in the laboratory (fig. 7). It is a machine that spins at high speed. Centrifugal force causes substances of

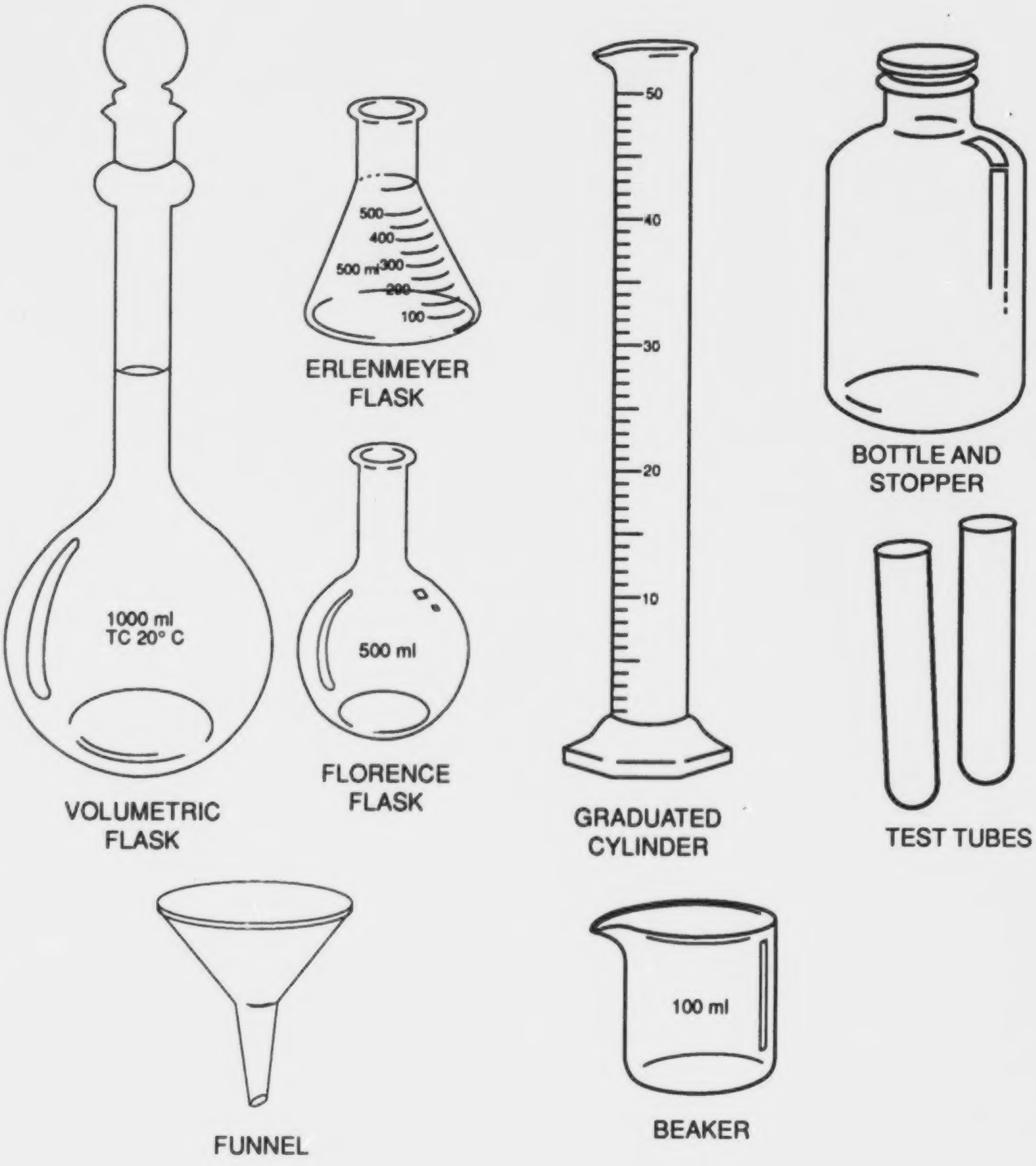

ERLENMEYER FLASK
500
400
500 ml
300
200
100
VOLUMETRIC FLASK
1000 ml
TC 20° C
FLORENCE FLASK
500 ml
GRADUATED CYLINDER
50
40
30
20
10
BOTTLE AND STOPPER
TEST TUBES
FUNNEL
BEAKER
100 ml

Fig. 2. Glassware

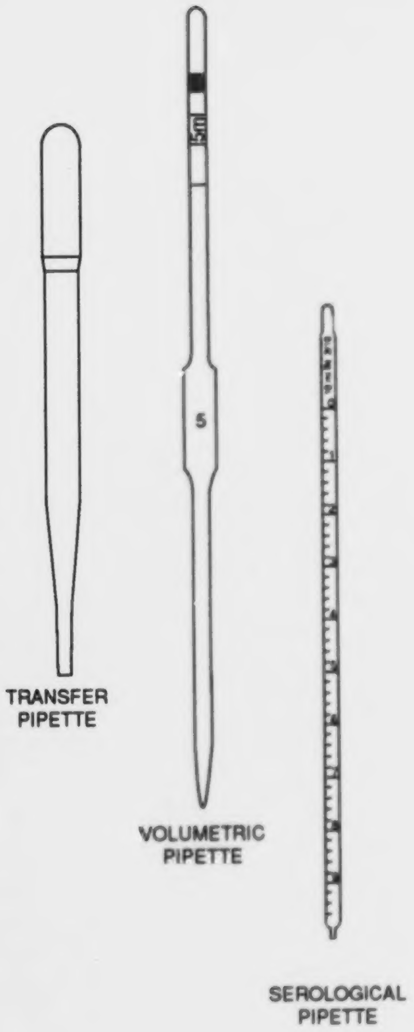

TRANSFER PIPETTE
5
VOLUMETRIC PIPETTE
SEROLOGICAL PIPETTE

Fig. 3. Types of pipettes

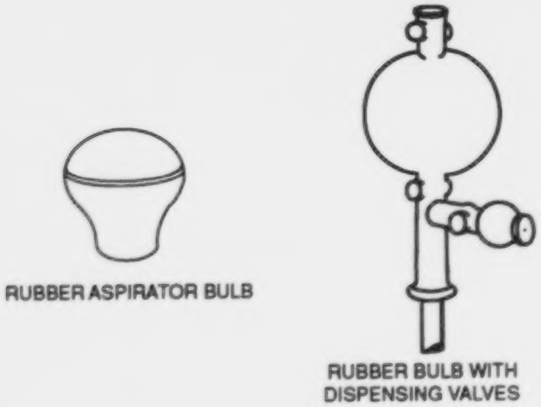

RUBBER ASPIRATOR BULB
RUBBER BULB WITH DISPENSING VALVES

Fig. 4. Types of bulbs

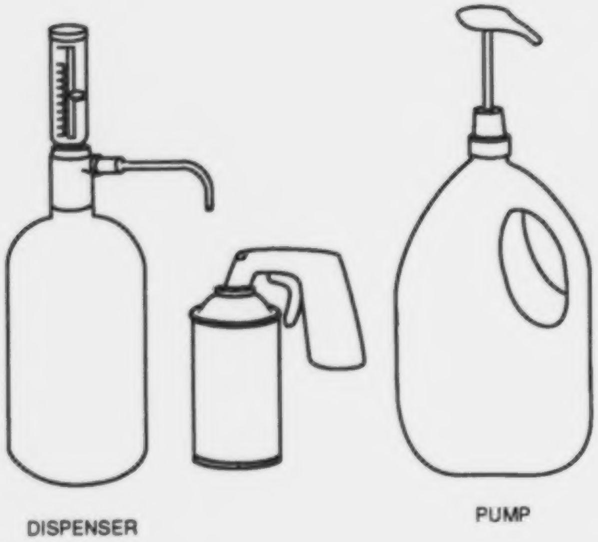

DISPENSER
PUMP

Fig. 5. Pumps and dispensers for reagents

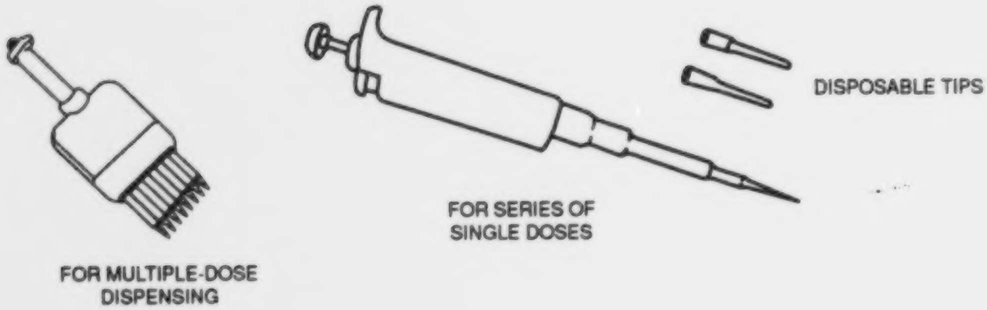

FOR MULTIPLE-DOSE DISPENSING
FOR SERIES OF SINGLE DOSES
DISPOSABLE TIPS

Fig. 6. Multiple test dispensing devices

different densities to separate. Heavier particles fall to the bottom. Lighter particles and fluids collect above. Laboratory centrifuges are used in the separation of serum or plasma from whole blood, bacteria from liquid media, and to concentrate the sediment in urine for microscopic examination.

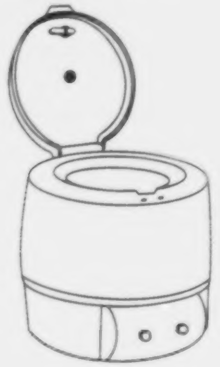


Fig. 7. Centrifuge

Centrifuges vary a great deal in size, but they must all be used with great care. Table models should be placed on a sturdy bench or table where there is plenty of room for the machine to spin. They should not be placed near the more delicate or sensitive equipment that might be affected by the vibration of the centrifuge. Large models are usually bolted into concrete on the floor.

Any items put in the centrifuge must be placed so that they balance each other. For example, if only two test tubes are used, they must be of the same weight and placed exactly opposite each other. In using large centrifuges, specimens are weighed to be sure they balance. This helps to avoid damage to the centrifuge, breakage of specimen tubes, and danger to personnel. The lid of the centrifuge must be securely closed before the machine is turned on. Speed should be accelerated slowly.

After the centrifuge has been turned off, it will stop spinning gradually. Most machines have a brake to be used if needed. Do not use the brake to stop the machine just because you are in a hurry and do not want to wait until the machine stops spinning. *Never stop the machine with your hand.* You may injure yourself, the specimens, the centrifuge, or all three! If breakage occurs while the machine is running, quickly turn off the power supply, but never open it until it is completely stopped.

The inside and outside of the centrifuge should be kept free of dirt with mild detergent and water. Spills should be cleaned at once. The machine should be left covered with the lid, when not in use, to keep dirt and debris out. You may use a test tube brush to clean test tube carriers. If breakage should occur, you must remove all fragments and clean all parts at once. If the centrifuge needs repair, check the operating manual for repair suggestions and for the name and address of repair personnel.

A special type of centrifuge is used in performing a *microhematocrit.* It is specifically designed to hold the capillary tubes required for the test. The purpose of a microhematocrit test is to determine an accurate packed cell volume of red blood cells using small quantities of blood. The centrifuge operates at very high speeds, from 11,000 to 15,000 rpm. The machines are usually equipped with an automatic timer because an exact running time is required to produce constant test results. The accuracy of the timer should be checked at frequent intervals with a stopwatch or an electric clock.

A *refrigerated centrifuge* is exactly what the name implies. It is a centrifuge cooled by mechanical refrigeration with a thermostat to keep the temperature constant. This protects heat-sensitive specimens from the heat generated by the centrifugal force of the spinning. These are used in some blood banks and in the preparation of biological specimens.

Tabletop centrifuges are used in serological testing to improve red blood cell antigen-antibody reaction and also to wash cell suspensions. One type of centrifuge has fixed speed and variable time. The other has both variable speed and variable time. The routine checks of *revolutions per minute* (rpm) and time of centrifugation are absolutely essential for consistent results that meet quality-control standards.

Variable-speed centrifuges have a relative setting indicator as part of their speed control. To set the speed control, a tachometer is used to determine the number of revolutions it makes per minute. Then the speed is marked as such. From time to time the laboratory will reverse the procedure. The control is set, and the tachometer is read to see if the specified revolutions are actually being produced. Tachometers may be the strobe or vibrating-reed type.

Centrifugation time is checked with a stopwatch. Time is checked from the beginning of acceleration to the point of deceleration.

Acceleration time is more difficult to control because it must be varied according to the thickness (viscosity) and volume of the suspending liquid through which the cells must pass in the tube. Therefore, it is necessary to calibrate the centrifuges for all the different kinds of tests that are performed.

Each centrifuge should be calibrated to determine the speed and time that produces agglutination without causing false positive reactions. Centrifugation causes the cells to come together in a well-defined button. However, centrifugation should not pack the cells so tightly that unagglutinated cells cannot be resuspended with gentle agitation. A clearly delineated cell button is a small round dot surrounded by clear supernatant fluid. The outer surface of the cell button should be clear and crisp, not jagged or fuzzy.

When the proper setting for centrifugation is determined for the kind of test to be performed, the supernatant fluid will be clear, the cell button well be clearly delineated, and it will resuspend with very gentle agitation. The final time that is selected must show the strongest reaction with the positive cell, as well as a clear-cut negative reaction with the negative cell. The final calibration selection should be marked on the centrifuge (along with the date the calibration was performed) so that everyone will use the proper settings.

To check the speed control using a *stroboscope*, put a transparent heavy plastic lid on machines that have metal covers. Place a mark on the centrifuge shaft, and start the motor. Turn the strobe light to "ON," and adjust until the mark appears to stand still. At this point, read the speed of rotation from the stroboscope dial. Check variable-speed centrifuges at several dial settings, both loaded and unloaded. Compare the speeds with those listed on the calibration chart for that centrifuge.

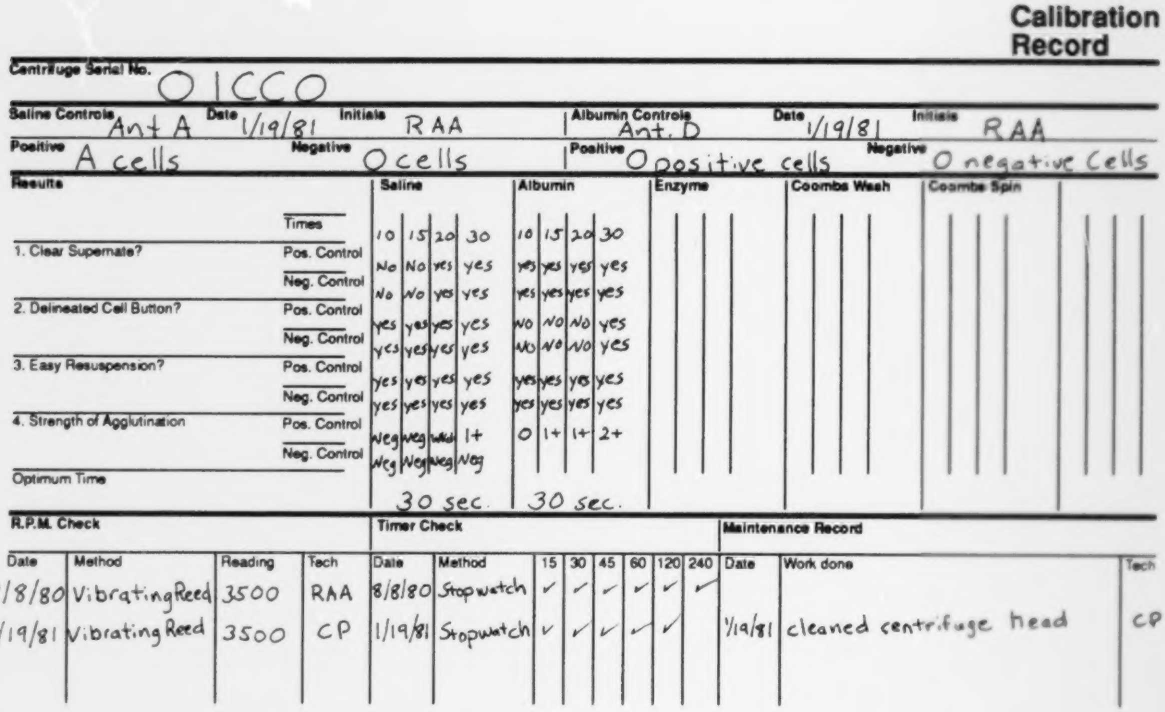
It is perhaps easier to check the speed control by using a vibrating-reed type of tachometer because this simply attaches to or is held against the casing while the centrifuge is running. Reeds of various resonating frequencies are used in miniature tuning forks. Each one vibrates as the centrifuge reaches the revolutions needed to activate it. Record the speed of revolution as it is determined.

To calibrate centrifuges, follow this procedure. (1) Use serum that contains an antibody that will produce a 1+ agglutination when viewed with the unaided eye (macroscopically). Commercial antiserum may be diluted with bovine albumin or human group AB serum to produce a satisfactory protein concentration. Some laboratories use anti-Rho (D) that is diluted about 1:25 for a 1+ reaction with Rh-positive cells. (2) Select a negative sample. Prepare a suspension of fresh red blood cells in the concentration that is used in routine testing (2% to 5%).

A. *For saline-active antibodies:*
   Serum for group A (anti-B) is diluted to produce 1+ agglutination.
   Positive control: group B red cells in suspension.

B. *For albumin-active antibodies:*
   Anti-Rho (D) is diluted to produce 1+ agglutination.
   Positive control: positive red cells in suspension.
   Negative control: Rh-negative red cells in suspension.

For each medium (saline, albumin, etc.), prepare five 10 x 75 or 12 x 75 mm tubes for positive reactions and the same for negative reactions. Add serum and test cells to each tube just before centrifugation. In pairs (a positive and a negative), centrifuge the tubes for different times (10, 15, 20, 30, 45, and 60 seconds). Observe each tube for agglutination, and record results as shown in table 1.



Calibration Record

Centrifuge Serial No. O ICCO

Saline Controls Ant A Date 1/19/81 Initials RAA | Albumin Controls Ant. D Date 1/19/81 Initials RAA

Positive A cells Negative O cells | Positive O positive cells Negative O negative Cells

Results		Saline				Albumin				Enzyme	Coombs Wash	Coombs Spin	
	Times	10	15	20	30	10	15	20	30				
1. Clear Supernate?	Pos. Control	No	No	yes	yes	yes	yes	yes	yes				
	Neg. Control	No	No	yes	yes	yes	yes	yes	yes				
2. Delineated Cell Button?	Pos. Control	yes	yes	yes	yes	No	No	No	yes				
	Neg. Control	yes	yes	yes	yes	No	No	No	yes				
3. Easy Resuspension?	Pos. Control	yes	yes	yes	yes	yes	yes	yes	yes				
	Neg. Control	yes	yes	yes	yes	yes	yes	yes	yes				
4. Strength of Agglutination	Pos. Control	Neg	Neg	wk	1+	0	1+	1+	2+				
	Neg. Control	Neg	Neg	Neg	Neg								
Optimum Time		30 sec.				30 sec.							

R.P.M. Check				Timer Check								Maintenance Record		
Date	Method	Reading	Tech	Date	Method	15	30	45	60	120	240	Date	Work done	Tech
8/8/80	Vibrating Reed	3500	RAA	8/8/80	Stopwatch	✓	✓	✓	✓	✓	✓			
1/19/81	Vibrating Reed	3500	CP	1/19/81	Stopwatch	✓	✓	✓	✓	✓		1/19/81	cleaned centrifuge head	CP

Fig. 8. Calibration record

The optimum time of centrifugation is the least time required to fulfill all of the criteria. Since, in table 1, the 45-second and 60-second spins are both adequate, the optimum time for this centrifuge is 45 seconds.

Figure 8 shows an actual sample of a calibration record from a Texas hospital laboratory.

The Microscope

The *microscope* is used to magnify very small objects. There are many kinds of micro-

Table 1

Criteria	*Time in Seconds* 10	15	20	30	45	60
Clear supernatant	no	no	yes	yes	yes	yes
Delineated cell button	no	no	no	no	yes	yes
Cells easily resuspended	yes	yes	yes	yes	yes	yes
Negative reactions clear	yes	yes	yes	yes	yes	yes
Degree of agglutination	0	0	wk+	1+	1+	1+

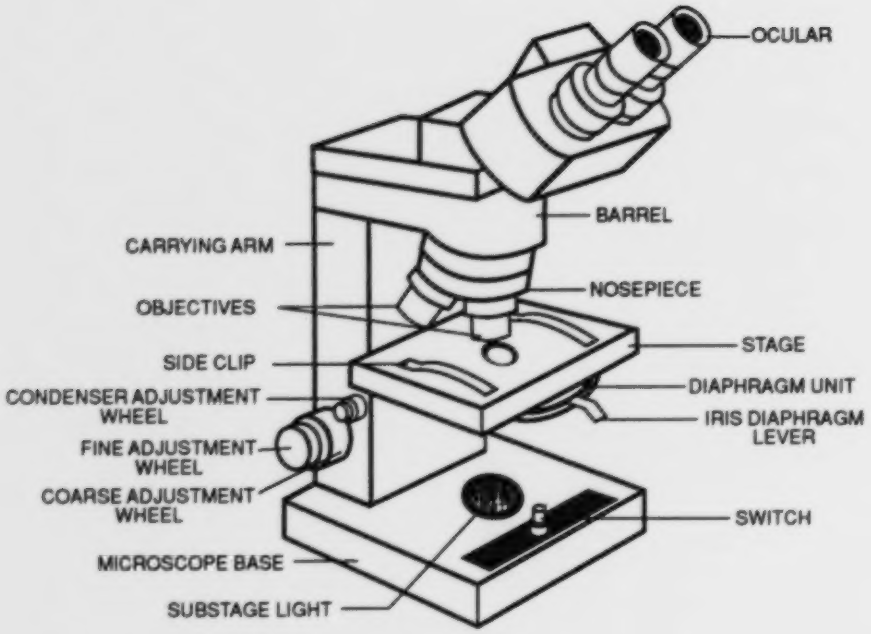

OCULAR
BARREL
CARRYING ARM
NOSEPIECE
OBJECTIVES
STAGE
SIDE CLIP
DIAPHRAGM UNIT
CONDENSER ADJUSTMENT WHEEL
IRIS DIAPHRAGM LEVER
FINE ADJUSTMENT WHEEL
COARSE ADJUSTMENT WHEEL
SWITCH
MICROSCOPE BASE
SUBSTAGE LIGHT

Fig. 9. Parts of the microscope

scopes. They are designed for different specific uses and vary in price. The first microscopes were simple microscopes. A ***simple microscope*** is merely a single lens or magnifying glass. Some simple microscopes can magnify 200X. A ***compound microscope*** is a series of lenses to increase magnification and will magnify up to 1,000X.

If the microscope has a single eyepiece (ocular), it is ***monocular***. These microscopes are commonly used to teach students how to use the microscope. ***Binocular*** microscopes (two eyepieces) are used in most laboratories. ***Teaching*** microscopes have two sets of eyepieces (four total) so the instructor and student may see the same field at the same time.

A ***darkfield*** microscope is used in the identification of some bacteria. In this microscope, a special condenser causes light to reflect from the specimen onto a dark background. Thus, you see a light object in a dark field.

The ***electron*** microscope is used when the greatest magnification is required, such as the study of viruses. Magnifications of 400,000X are possible with this microscope.

Other kinds of microscopes used for special studies are the ***polarization*** microscope, the ***ultraviolet*** microscope, and the ***phase contrast*** microscope.

Parts of the Microscope

A compound microscope has the following parts, as shown in figure 9.

- The ***eyepiece (ocular)*** contains a magnifying lens.
- The ***body tube*** or ***barrel*** connects the eyepiece to the objectives.
- The ***nosepiece*** supports the objectives.
- The ***objectives*** contain lenses for magnification. The ***oil-immersion objective*** is a special objective on some microscopes.

It is designed to be used with a drop of immersion oil between the object and the surface of the lens.

- The *arm* is the support by which the microscope is carried.
- The *stage* is the part of the microscope where the slide is placed.
- The *slide clips* hold the glass slide in place on the stage.
- The *substage control* moves the stage for focusing but is not found on all microscopes.
- The *diaphragm* makes possible the adjustment of the amount of light entering the objectives.
- The *mirror* reflects light rays into the objective. Instead of a mirror, most microscopes used in the medical laboratory have a *substage lamp* to provide light.
- The *coarse adjustment wheel* moves either the stage or objective maximally for the first sighting of the object.
- The *fine adjustment wheel* moves either the stage or objective minimally for fine focusing.
- The *base* supports the body of the microscope.

Use of the Microscope

One of the most important skills the medical laboratory worker must learn is the use of the microscope. The correct procedure is given in the following steps.

Turn the eyepiece until the low-power objective is in place. You will feel and hear a click.

Then adjust the mirror or substage lamp with the eye at the eyepiece. If you are using a mirror, the flat side should face the stage if a condenser is used. Use the concave side if no condenser is present. Use the diaphragm to control the amount of light.

Now place the prepared slide on the stage. Using the coarse adjustment wheel, move the tube or barrel downward until the low-power objective is close to the slide. Be sure to watch the tube move as you turn the coarse adjustment wheel. Never focus downward with the coarse adjustment wheel while peering through the eyepiece. This will avoid breaking the slide, cover slips, and even lenses.

Place your eye at the eyepiece, and gradually move the tube upward, using the coarse adjustment wheel, until you see the object on the slide. If you fail to find the object, start over. Lower the tube again, and gradually raise it. After you have found the object using the coarse adjustment wheel, use the fine adjustment wheel to get a sharper image. You may need to readjust the light source and diaphragm to get the best image.

Once you have focused clearly using the low-power objective, you can change to the high-power objective. The image should stay in focus, but you will need to use the fine adjustment wheel to maintain a clear image.

The magnification of the object viewed is the product of the magnification of the eyepiece multiplied by the magnification of the objective used.

Example: Eyepiece is 10X, and objective is 10X.
Magnification will be 100X.

When you have finished with the microscope, you must remove the slide. Then clean the eyepiece, objectives, and stage with lens paper. Put the low-power objective in place, and focus all the way down with the coarse adjustment wheel. The microscope should be covered or housed in a closed case when not in use.

To clean the microscope, you use lens paper. Xylol (xylene) may be used to remove oil from oil-immersion lenses. Since xylol is toxic, it should be used sparingly and in well-ventilated areas.

Always use both hands to carry a microscope. You should put one hand on the carrying arm and the other under the base of the instrument. Never try to carry other objects while carrying the microscope.

If the microscope does not function properly in any way, do not try to force it or fix it, but notify your supervisor at once.

The Autoclave and the Oven

The pieces of equipment used for sterilization in the laboratory are the hot air oven and the autoclave. The choice of method depends on the type of material to be decontaminated and the equipment available.

The hot air oven provides dry heat used to sterilize glassware or metal instruments. A temperature of 160°C for two hours is usually used.

The autoclave sterilizes by the use of steam under pressure. It is used to sterilize glassware, sharp-edged or hinged instruments, water, culture media, or linens. Glassware is sterilized at 121°C for 15 to 20 minutes at a pressure of 15 lb/sq in. The autoclave is programmed with automatic timed cycles. Time and temperature are set to kill viruses, fungus, and bacteria including spore forms. Autoclaving is used to decontaminate biohazardous materials such as blood specimens and bacterial cultures before they are disposed of finally.

Constant Temperature Appliances

A *water bath* is simply a container of water maintained at a constant, fixed temperature (fig. 10). It is used for some laboratory tests in which specimens must be incubated at a given temperature. The temperature of the bath is controlled by a heating device and a thermostat. Some water baths have a pump to circulate the water. Be sure that the pump housing and heater are always immersed in water.

A *heating block* is a solid metal block on which containers may be set or with holes suitable for holding various sizes of test tubes (fig. 11). It has a heating device and a thermostat so a constant temperature may be maintained.

Specimens incubated in a water bath emerge dripping with water. A heating block eliminates this. However, foreign materials from spillage and breakage tend to accumulate in the test tube holes of the heating block. Some particles may be removed by simply turning the block upside down and shaking or by swabbing the holes. For thorough cleaning, remove the top plate.

Many other kinds of laboratory equipment must maintain a specific constant temperature. For example, it is critical that the temperature of bacteriology incubators, and of ovens used for drying samples, evaporation, or sterilization, be accurately controlled.

Summary

This unit deals with the storage and use of equipment in the laboratory. The term *calibration* is defined and discussed. The use of the following glassware is described—graduated cylinders, pipettes, flasks, beakers, and test tubes. Large pieces of equipment are listed and explained. These include centrifuges, microscopes, ovens, autoclaves, water baths, and heating blocks. Quality control of test results can be maintained through accurate calibration and maintenance of equipment.

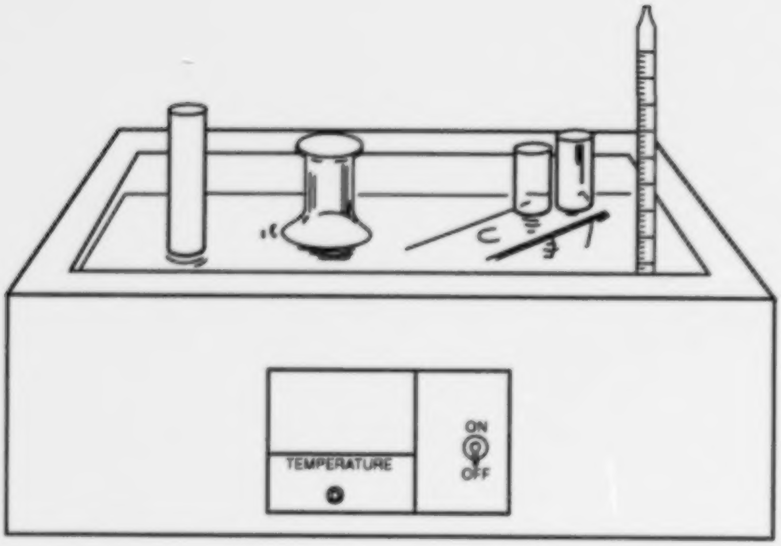

ON
TEMPERATURE
OFF

Fig. 10. Water bath

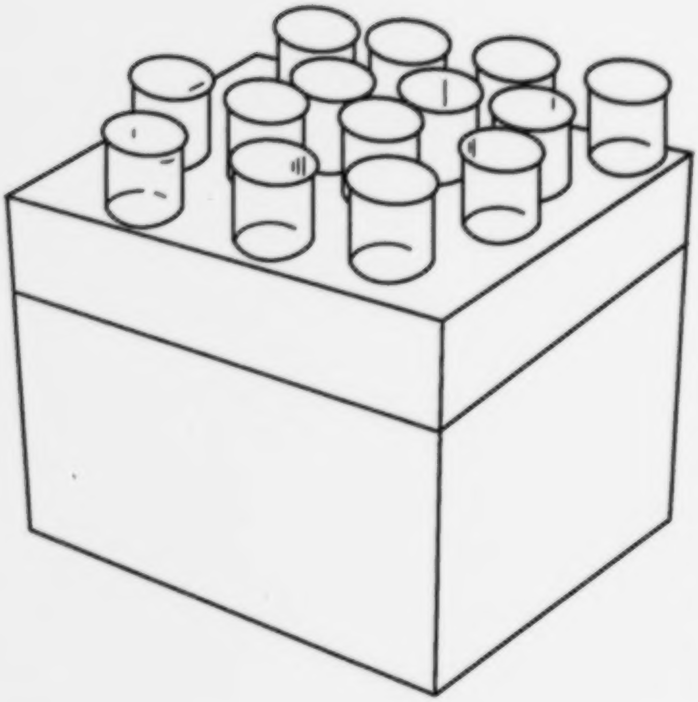


Fig. 11. Heating block

5

Urinalysis

Objectives: After completing this unit, you will be able to—

1. *give directions for collection of urine specimens;*
2. *label urine specimens;*
3. *perform macroscopic examinations, and record physical characteristics;*
4. *perform specific gravity determinations;*
5. *interpret "stix" and record readings of pH, protein, glucose, ketones, bilirubin, urobilinogen, blood, and nitrite; and*
6. *examine urine specimens microscopically.*

Terms and Definitions

amorphous—without a definite shape.

anuria—absence of excretion of urine from the body; failure of kidneys to function.

artifact—an artificial structure.

casts—cylindrical structures with blunt ends and parallel sides and composed of mucoprotein.

convoluted—rolled together or coiled.

decant—pour off liquid so sediment remains in bottom of container.

hematuria—presence of blood in the urine.

macroscopic—visible to the unaided eye.

microscopic—visible if using a microscope; not visible to the unaided eye.

occult blood—minute amount of blood which is detected by chemical tests or microscopic examination.

polyuria—excessive urination.

pyuria—excessive white blood cells (pus) in the urine.

quantitative—test that determines how much of a substance is present as opposed to *qualitative*, which just indicates that a substance is present.

sediment—substance that separates out of solution and settles to the bottom.

specific gravity—weight of a substance as compared with an equal volume of water at the same temperature.

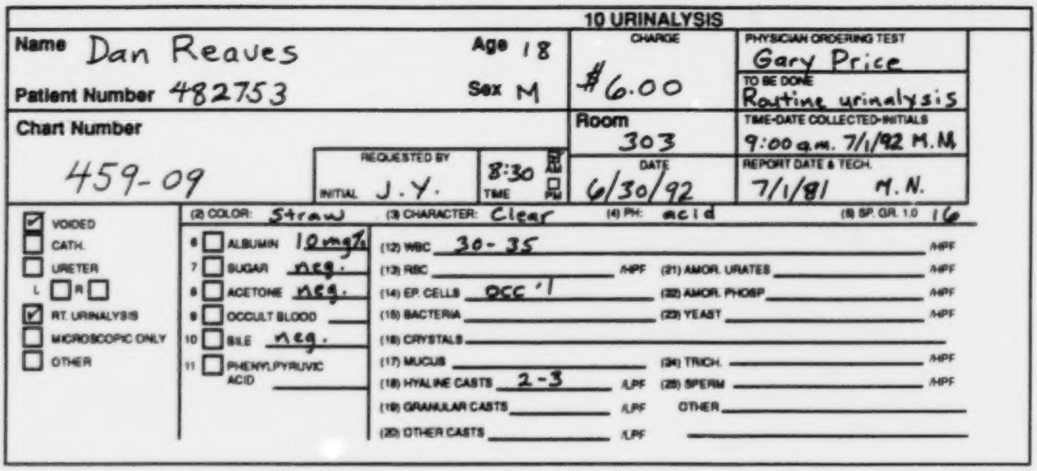
void—urinate.

The urine is a very complex aqueous solution of several organic and inorganic substances, most of which are either waste products from body metabolism or products formed directly from the foods eaten. The most important organic materials are urea, uric acid, and creatinine. The chief inorganic ones are chlorides, phosphate, sulfates, and ammonia.

Certain materials appear in the urine only when disease conditions are present. These are proteins, carbohydrates, acetone, bile, and hemoglobin. Examinations should always be carried out on specimens that are fresh because the chemical composition is altered upon standing. Urine that cannot be examined immediately should be refrigerated.

Collecting the Specimen

Routinely, laboratories process urine specimens collected either as the first morning specimen or collected randomly. The *first morning specimen* is preferred because of the concentration. It is collected outside the laboratory by the patient or the nursing service. A *random specimen* is collected any time and has a variable dilution. If the patient collects the specimen at home, be certain that the laboratory provides the container. Patients must not be allowed to use dirty receptacles.



10 URINALYSIS

Name Dan Reaves
Age 18
Patient Number 482753
Sex M
CHARGE #6.00
PHYSICIAN ORDERING TEST Gary Price
TO BE DONE Routine urinalysis
Chart Number 459-09
REQUESTED BY INITIAL J.Y.
TIME 8:30 ☑ AM ☐ PM
Room 303
DATE 6/30/92
TIME-DATE COLLECTED-INITIALS 9:00 a.m. 7/1/92 M.M.
REPORT DATE & TECH. 7/1/81 M.N.

☑ VOIDED
☐ CATH.
☐ URETER
L ☐ R ☐
☑ RT. URINALYSIS
☐ MICROSCOPIC ONLY
☐ OTHER

(2) COLOR: Straw
(3) CHARACTER: Clear
(4) PH: acid
(5) SP. GR. 1.0 16

6 ☐ ALBUMIN 10 mg%
7 ☐ SUGAR neg.
8 ☐ ACETONE neg.
9 ☐ OCCULT BLOOD
10 ☐ BILE neg.
11 ☐ PHENYLPYRUVIC ACID

(12) WBC 30-35 /HPF
(13) RBC /HPF
(14) EP. CELLS occ'l
(15) BACTERIA
(16) CRYSTALS
(17) MUCUS
(18) HYALINE CASTS 2-3 /LPF
(19) GRANULAR CASTS /LPF
(20) OTHER CASTS /LPF
(21) AMOR. URATES /HPF
(22) AMOR. PHOSP. /HPF
(23) YEAST /HPF
(24) TRICH. /HPF
(25) SPERM /HPF
OTHER

Fig. 1A. Example of completed urinalysis request forms—handwritten

In the hospital, the collection of routine urine specimens is the responsibility of the nursing service. They collect, label, provide the request form, and deliver the specimen to the laboratory. The laboratory personnel are then responsible for checking for complete labeling and agreement between the container and the request slip. Then they assign a laboratory number and log the specimen if required.

Sometimes, a midstream or "clean-catch" urine specimen is preferred. This means the specimen is essentially free of contamination. The patient is instructed to clean the urethral area if female, or glans if male. In the hospital, nursing personnel will assist the patient. The patient then begins to void in the toilet. This rinses the urethra. The midstream urine is collected in a clean container. The remaining urine is voided into the toilet. If the midstream specimen is to be used for bacteriologic studies, a sterile container must be used.

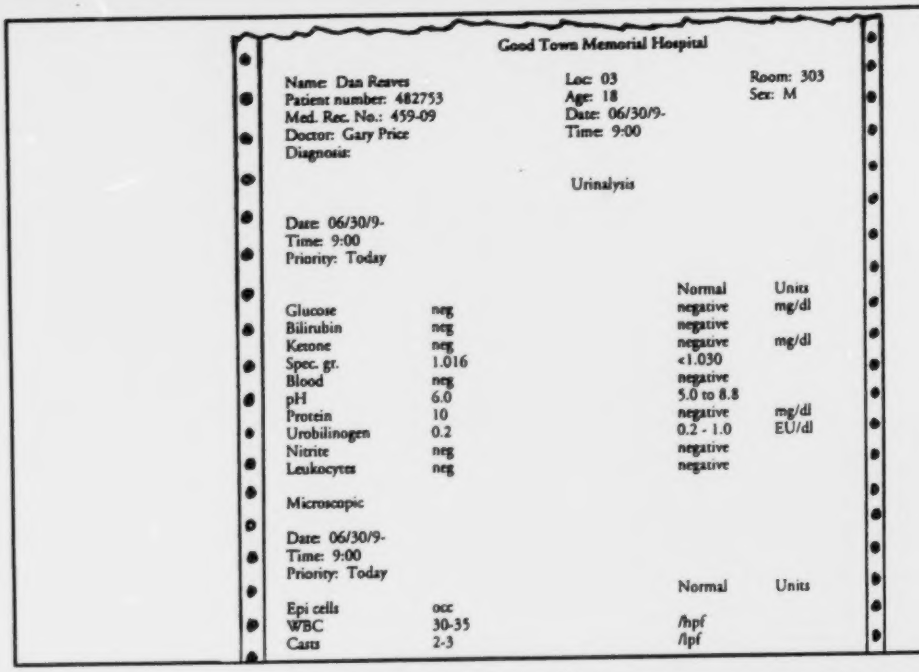
Rarely, catheterization is used to obtain clean urine specimens for bacteriologic study. Such procedures are infrequently ordered because the use of the catheter may cause urinary tract infections.

In a clinic or doctor's office, the medical laboratory worker may be responsible for urine specimen collection. The following procedure may be used:

1. Provide a clean, dry container.
2. Ask the patient to provide a urine specimen.
3. Tell the patient the location of the rest room and where the collected specimen is to be placed.
4. When the specimen is returned, label it properly, and provide the proper report form.
5. Perform the tests as soon as possible. If there must be a delay, refrigerate the urine specimens. Bacteria multiply rapidly, and leukocytes and casts tend to disintegrate at room temperature.

Figure 1 shows correctly completed urinalysis request forms, one done by hand (fig. 1A) and one a computer printout (fig. 1B). They are based on the following information:

1. The patient's name is Dan Reaves, 18 years old, room 303; chart #459-09, Dr. Gary Price. Ward clerk Janie Yates filled out the request slip at 8:30 A.M. on 6/30/92 to be done in A.M. 7/1/92. Voided urine was collected at 9:00 A.M. by nurse aide Mary Moore and taken to the laboratory for routine urinalysis.
2. Mary Neal performed the urinalysis on 7/1/92 and did a microscopic examination on the centrifuged sediment with these findings: The urine was straw-colored and clear. It had a specific gravity of 1.016 with an acid pH. The reagent strip indicated 10 mg% albumin. Tests for sugar, acetone, and bile were negative. Microscopically, there were 30-35 white blood cells/high-power field, 2-3



Good Town Memorial Hospital

Name: Dan Reaves
Patient number: 482753
Med. Rec. No.: 459-09
Doctor: Gary Price
Diagnosis:

Loc: 03
Age: 18
Date: 06/30/9-
Time: 9:00

Room: 303
Sex: M

Urinalysis

Date: 06/30/9-
Time: 9:00
Priority: Today

		Normal	Units
Glucose	neg	negative	mg/dl
Bilirubin	neg	negative	
Ketone	neg	negative	mg/dl
Spec. gr.	1.016	<1.030	
Blood	neg	negative	
pH	6.0	5.0 to 8.8	
Protein	10	negative	mg/dl
Urobilinogen	0.2	0.2 - 1.0	EU/dl
Nitrite	neg	negative	
Leukocytes	neg	negative	

Microscopic

Date: 06/30/9-
Time: 9:00
Priority: Today

		Normal	Units
Epi cells	occ		
WBC	30-35	/hpf	
Casts	2-3	/lpf	

Fig. 1B. Example of completed urinalysis request form—computer printout

hyaline casts/low-power field, and an occasional epithelial cell. The fee was $6.00.

Urine specimens may be labeled to indicate the patient's name, the doctor ordering the test, the date collected, and whether routine or special tests are requested. Labels may also include the age of the patient and the chart number or hospital room number (see fig. 2).

There are several different types of urine specimen containers (see fig. 3). Most hospitals use plastic disposable containers. Reusable plastic, glass, or metal containers are commonly used in clinic laboratories.

Performing the Urinalysis

Once the urine has been collected, properly labeled, and delivered, the laboratory is ready to begin the urinalysis. This is probably the most common laboratory procedure and consists of a series of tests performed on urine. A ***routine urinalysis*** is that series of tests selected by a particular institution to be performed on most urine specimens. It generally includes both macroscopic and microscopic examinations, as well as chemical tests and measurement of specific gravity.

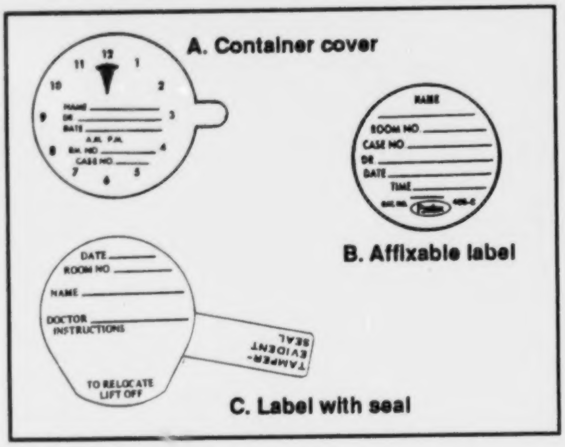


Fig. 2. Samples of labels for urine specimens

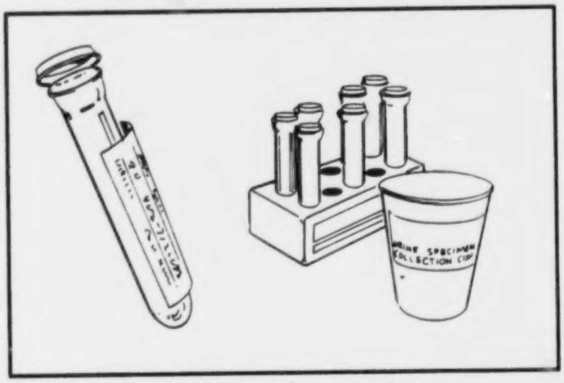


Fig. 3. Urine specimen containers

Macroscopic Examination

Macroscopic examination is noted without the aid of a microscope and generally includes color and character. *Color* varies with concentration, urine pigments, and the presence of metabolic products. These common terms are used to describe the color of urine:

colorless—very diluted
straw—almost colorless
yellow—more concentrated; medications
orange—very concentrated; carotene, bile, or medications present
green or blue green—dyes or medications
pink, red, or dark reddish orange—blood, hemoglobin, porphyrin, bacteria, vegetables, medicine, or normal pigments after pH change
black or brown—bilirubin, iron, porphyrin, melanin, or medications

Character is the appearance or transparency of the urine and is reported as clear, partly cloudy (due to crystals, WBCs, or blood), cloudy (many crystals, WBCs, blood), or grossly bloody. The odor of the urine is not routinely reported unless it is particularly unusual.

Specific Gravity

The *specific gravity* is the weight (mass) of a measured volume of urine compared with the same volume of water at the same temperature. The specific gravity of water is 1.000. Normal ranges of urine specific gravity (sp. gr.) are 1.010 to 1.020. However, samples of urine taken at random may be far above or below these figures. Generally, the specific gravity is inversely proportional to the quantity of urine. In other words, a large quantity of urine would have a low specific gravity. Variations in specific gravity are of value in detecting whether the kidneys are functioning normally. It is low in cases of chronic nephrosclerosis, diabetes insipidus, and some functional nervous disorders. It is high in cases of fever and functional kidney disease, and is highest in diabetes mellitus. Dehydration and decreased fluid intake also yield an increased specific gravity.

The specific gravity is conveniently measured by using either a refractometer or a urinometer. The refractometer is a hand-held instrument that requires only a drop of urine (see fig. 4). It is especially useful when only a small quantity of urine is available for testing.

To use the refractometer, follow these steps:

1. Hold the instrument in a horizontal position.
2. Clean the surface of the cover plate and prism with lens paper. Wipe lightly to avoid scratching the soft optical glass.
3. Place a large drop of urine sample on the prism face, and close the cover plate.
4. Hold the instrument so that the prism window faces the light source.
5. Turn the eyepiece to provide a sharp focus of the scale.

The border line between the light and dark areas indicates the specific gravity of the urine. Always report the answer in four digits, such as 1.009 or 1.025.

When using the refractometer, always hold the instrument with the fingers close to the eyepiece, and hold the hinged prism cover against the surface with the thumb and forefinger. Do not grasp the prism section as this will raise the temperature and result in an incorrect reading.

When a *urinometer* is used to determine the specific gravity of urine, a larger amount of urine is required—usually about 15 ml. The urine should be mixed and then poured into the cylinder (see fig. 5). Place the urinometer float in the urine, and twirl it gently to be certain the float is free. It is also important that bubbles be removed from the surface of the urine. These are easily removed with a strip of filter paper. Read the bottom of the meniscus, and report the answer in four digits (e.g., 1.018). Make frequent checks with distilled water to determine the reliability of the urinometer or refractometer.

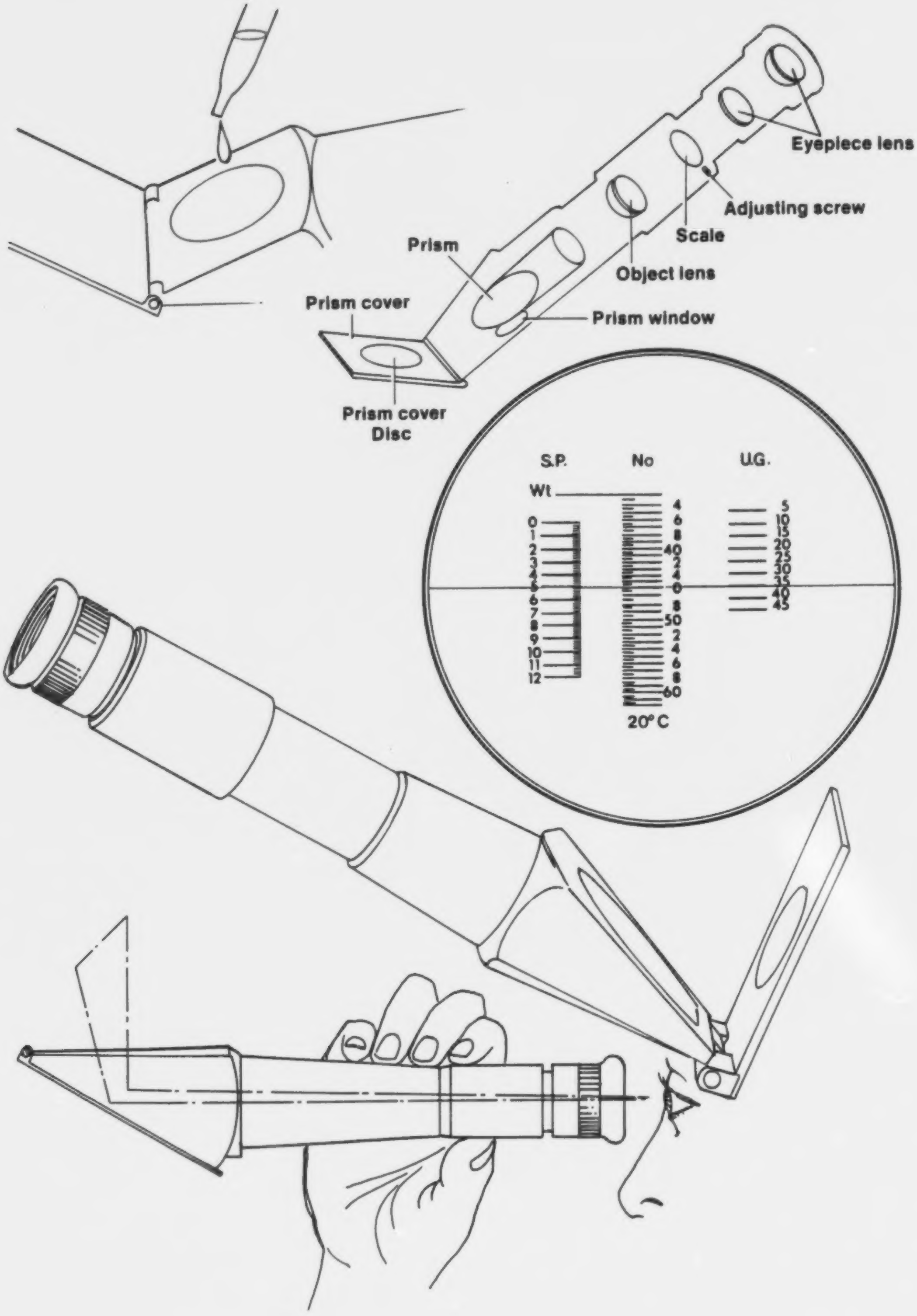

Eyepiece lens
Adjusting screw
Scale
Prism
Object lens
Prism cover
Prism window
Prism cover
Disc
S.P.
No
U.G.
Wt
0
1
2
3
4
5
6
7
8
9
10
11
12
4
6
8
40
2
4
6
8
50
2
4
6
8
60
20° C
5
10
15
20
25
30
35
40
45

Fig. 4. Refractometer or hand photometer

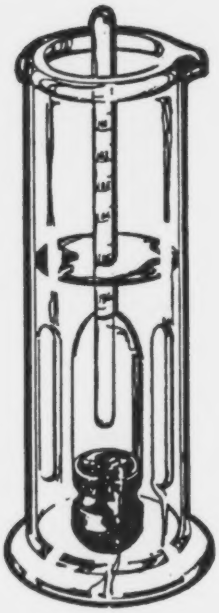


Fig. 5. Urinometer (float) inside cylinder

Determining pH

The acidity or alkalinity of the urine sample is referred to as the *pH.* Freshly voided urine is almost always acid, with a pH range of 4.8 to 7.0. Patients who eat high-protein diets may excrete more acid urine than those who consume mainly vegetables. A decreased pH is found in diabetic acidosis, fevers, pulmonary emphysema, diarrhea, dehydration, and during the use of some acidifying medications. Alkalinity (increase in pH) is found in chronic cystitis, certain genitourinary tract infections, pyloric obstruction, acute and chronic renal failure, and salicylate intoxication. Normally the urine becomes more acid with high-protein intake and sleep. It becomes more alkaline after collection and delay and after meals. The pH of the urine is easily determined using one of the commercial "stix" available. These provide a range of pH from 5 to 9 that includes a series of distinct color changes from orange to blue. The result is reported as acid, neutral, or alkaline.

Using Reagent Strips

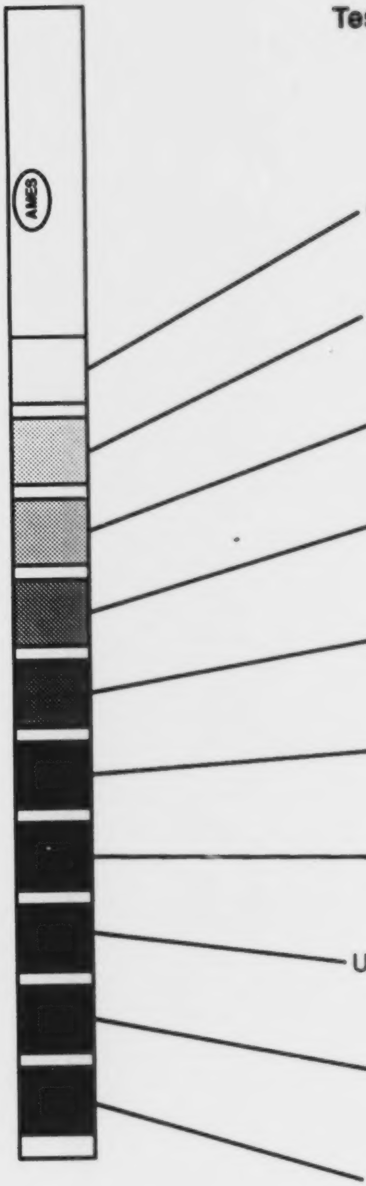
Chemical screening tests using "stix" are comprehensive, convenient, and economical. They give results that are reproducible. These are some of the more commonly used commercial multiple reagent strips that are manufactured and supplied by Ames Inc., Diagnostic Division. *Multistix* checks for pH, protein, glucose, ketone, bilirubin, blood, and urobilinogen. *Multistix 9* checks for all previous constituents plus nitrites and leukocytes. *Multistix 10 SG* checks for all constituents available on Multistix 9 plus a dip and read test for specific gravity (see fig. 6).

Also available commercially are single reagent tests: *Clinistix, Diastix,* and *Clinitest* all check for glucose, *Ketostix* tests for ketones, and *Ictotest* tests for the presence of bilirubin. All of these should be negative. Any protein, glucose, ketones, blood, bilirubin, urobilinogen, and nitrites present may indicate a pathological condition.

To use any of the reagent strips, dip the "stix" in the fresh urine. Tap the strip against the container side. Observe the specified times, and read accordingly. Compare with the color chart. Record test results. Every day, the reagent strips should be tested with controls to be certain that the strips are working. This ensures accuracy for quality control.

Microscopic Examination

The urine sample is now ready to be examined microscopically. Place 10 to 12 ml of well-mixed urine into a centrifuge tube, and centrifuge at 1,500 to 2,000 rpm for 5 minutes. Pour all of the urine out of the tube. Then place the tube in an upright position so that the fluid remaining on the sides flows to the bottom to dilute the sediment. Shake the sediment by tapping the tip of the tube on the hand. Place a small amount on a clean slide, and cover with a cover slip. If too much is poured onto the slide, the elements will be distributed through many layers and may be missed on examination. If



Test and Timing	Color Changes
Glucose, 30 seconds	Green to brown
Bilirubin, 30 seconds	Tan
Ketone, 40 seconds	Pink to purple
Specific gravity, 45 seconds	Blue for 1.000 through shades of green to 1.030 (gold)
Blood, 50 seconds	From orange through green to dark blue
pH, 60 seconds	Yellow—orange for acid pH to blue-green for alkaline pH
Protein, 60 seconds	Yellow for negative to green for positive
Urobilinogen, 60 seconds	Pinkish red
Nitrite, 60 seconds	Pink
Leukocytes, 2 minutes	From tan to purple

Fig. 6. Multistix 10SG reagent strip with test name, reading time, and change of color (Courtesy of Miles, Inc., Diagnostic Division, Elkhart, Indiana)

too little is poured onto the slide, the sediment may dry before the examination is completed. Some laboratories prefer to use stained sediment. If this is the case, add one drop of crystal-violet safranin stain (Sternheimer Stain) to the sediment, mix, and allow to stand for 3 minutes. Then place a drop on the slide, and cover with a cover slip.

Darken the field of the microscope by almost completely closing the diaphragm just beneath the stage. A well-darkened field is absolutely necessary if hyaline casts are to be found, because they are clear, colorless, and have low refractivity. First examine with the low-power objective, then with the high-power objective. Be careful to avoid careless transfer of sediment, use of too much light in the microscopic examination, use of high power only, drying of sediment, dirty equipment, and scratches on the slide.

Examine using low power for casts, epithelial cells, crystals, amorphous material, and mucous threads. Use the high power to examine for erythrocytes, leukocytes, and bacteria. Report all microscopic findings according to the reporting guide for your particular institution. Some institutions report as few, moderate, many. Others report in average numbers (average should include ten fields) per low- or high-power field, such as 6/lpf or 15/hpf. Others may report in pluses, such as +, ++, +++, and ++++ or 1+, 2+, 3+, and 4+.

Cellular Structures Found in Urinary Sediment

Cellular structures found in urinary sediment (fig. 7) include the following:

1. *Squamous epithelial cells*—these are large,

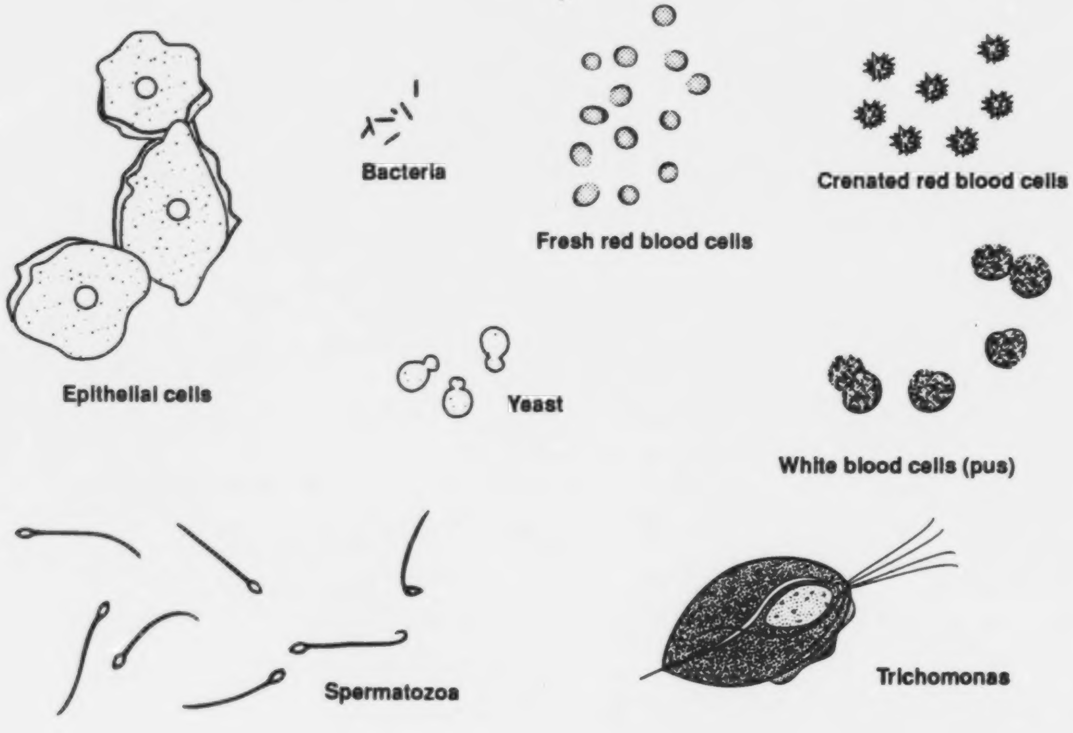


Fig. 7. Cellular structures found in urine sediment

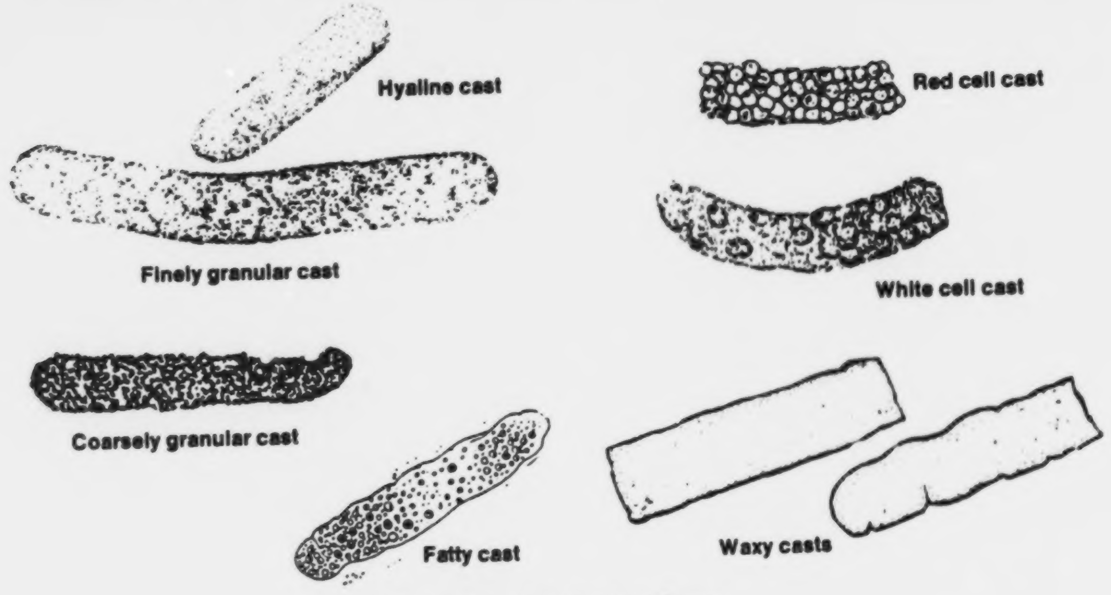


Fig. 8. Casts found in urine sediment

flat cells with small nuclei. They are derived from the urinary system, skin, or genitalia.

2. *Red blood cells (RBCs)*—these indicate bleeding in the urinary tract or contamination from menstrual flow in females. They may also be present in fever, or through injury during catheterization.

3. *White blood cells (WBCs)*—these may occur normally in females or children with 0-5/hpf in centrifuged specimens. Normal for males is 0-1/hpf. Increased numbers indicate infection.

4. *Bacteria*—these are not normally found in urine. They multiply rapidly when urine is left at room temperature. Their presence is significant if the collection is made using sterile technique.

5. *Spermatozoa*—their presence is not abnormal but should be reported.

6. *Trichomonas*—this is a parasite found in vaginal secretions. Their presence and frequency should be reported.

7. *Yeast*—these are single-celled oval organisms, not normally present.

Several kinds of *casts* may be found in urine sediment (fig. 8). The number is reported /lpf, but identification of type is confirmed on high power. Casts are cylindrical structures with blunt ends and parallel sides. They are composed of mucoprotein, and at times various other structures are included. Their presence indicates kidney disease. The type and frequency of cast is reported. These are some different kinds of casts found in urinary sediment: (1) *Hyaline casts* are cylindrical and transparent. (2) *Finely granular casts* contain fine granules. (3) *Coarsely granular casts* contain coarse granules. (4) *Red cell casts* are composed of RBCs. (5) *White blood cell casts* or *leukocyte casts* are composed mainly of leukocytes. (6) *Fatty casts* contain fat droplets. (7) *Waxy casts* are similar to hyaline casts, but are opaque and may be convoluted. (8) *Renal failure casts* are especially broad. They may be any of the previous types and may occur in large numbers called "showers."

Cylindroids resemble hyaline casts, but have tapered ends and sides that are not parallel. They may be precursors of casts. *Mucous threads* are long, irregular, transparent structures that may have tapered ends (see fig. 9).

Crystals Found in Acid Urine

These forms may be seen in acid urine (see fig. 10):

1. *Uric acid crystals*—shaped like plates, rhomboids, or clusters; may appear yellow or brownish.
2. *Amorphous urates*—may appear grossly as reddish sediment, and microscopically as

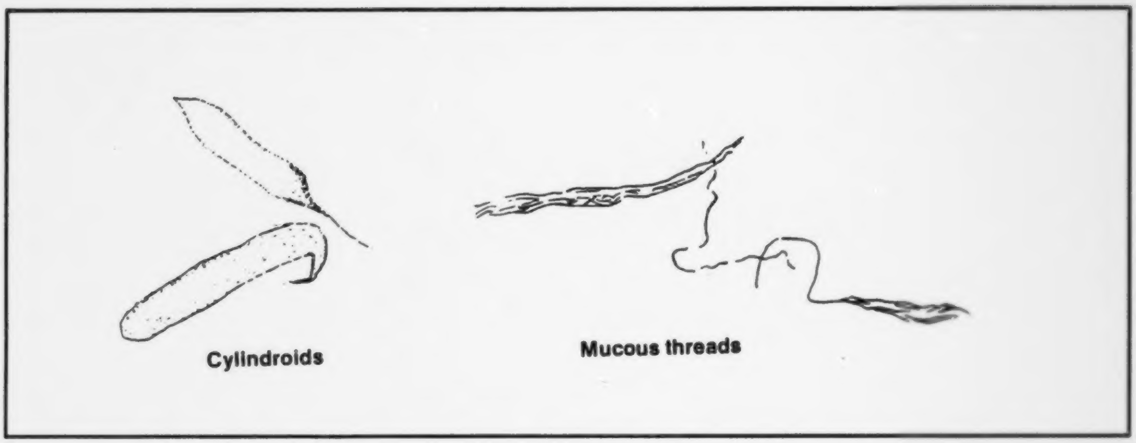

Cylindroids
Mucous threads

Fig. 9. Cylindroids and mucous threads

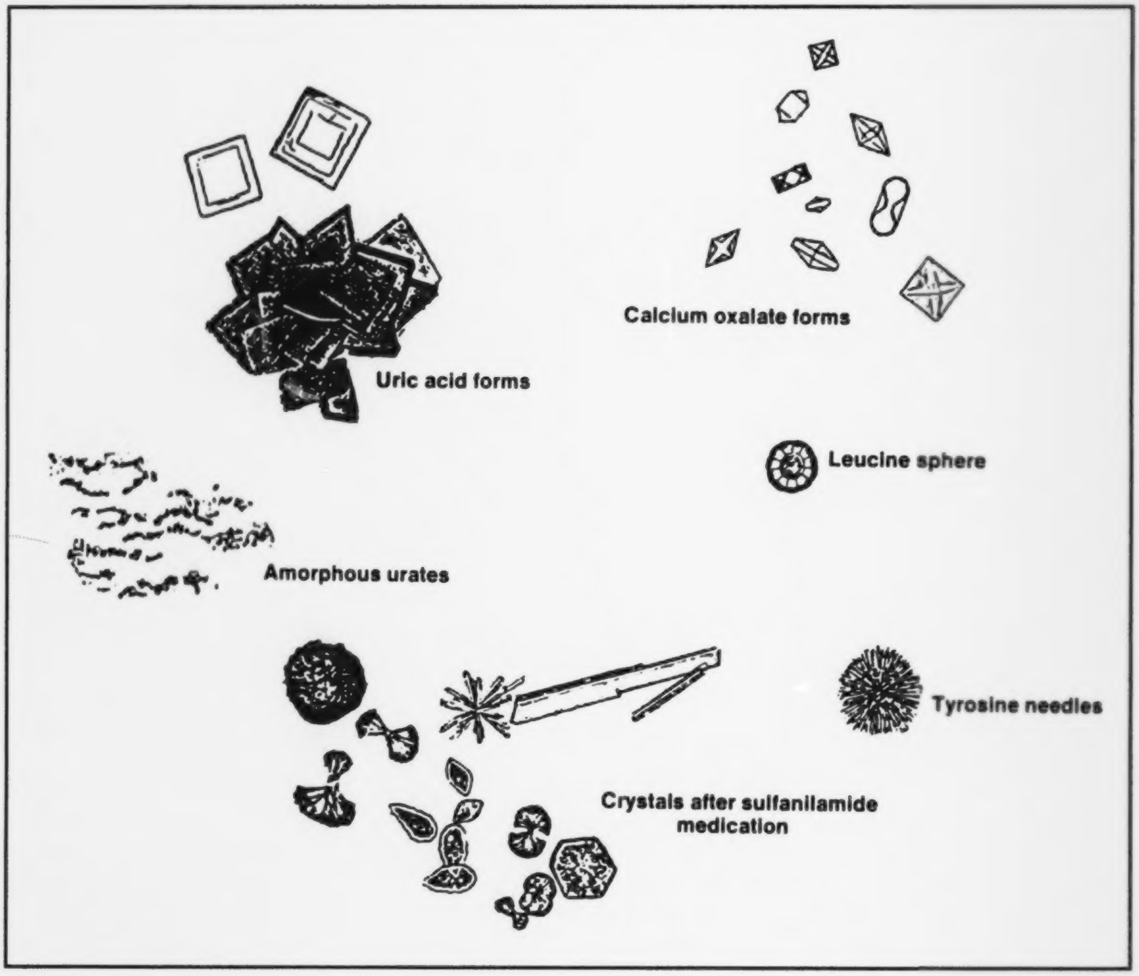

Calcium oxalate forms
Uric acid forms
Leucine sphere
Amorphous urates
Tyrosine needles
Crystals after sulfanilamide medication

Fig. 10. Crystals in acid urine

fine granules that are often so numerous they obscure all other structures. They may disappear upon heating or the addition of caustic soda.

3. *Calcium oxalate crystals*—commonly appear as oval spheres, biconcave disks, dumbbells, or as a crystal with a cross inside.
4. *Tyrosine crystals*—appear as yellowish, oily looking spheroids.
6. *Cystine crystals*—appear as flat, colorless, hexagonal plates with unequal sides.
7. *Sulfanilamide derivatives*—may produce crystals that appear as prisms, rhombic plates, or needles in sheaves, but all of these forms tend to have rounded edges rather than extreme pointed edges. If blood is also present, this may be a clue that internal crystallization is taking place.

Crystals Found in Alkaline Urine

Common sediments that appear in alkaline urine include the following (see fig. 11):

1. *Triple phosphate* or *ammoniomagnesium phosphate crystals*—colorless and usually occur as some modification of a prism with three, four, or six sides.
2. *Amorphous phosphates*—appear as numerous colorless granules similar to amorphous urates found in acid urine. They are soluble in acetic acid.
3. *Calcium phosphate crystals*—appear as long prisms or needles or as large irregular plates.
4. *Calcium carbonate crystals*—may be hidden among amorphous phosphate granules or may be colorless spheres or dumbbells.
5. *Ammonium biurate crystals*—these are yellow spheres covered with spicules and are sometimes called "thorn-apple crystals."

Fat globules may occur in either acid or alkaline urine. They appear as highly refractive globules of various sizes. These stain black with osmic acid, and orange or red with Sudan III or scarlet red.

To the inexperienced eye, talc and starch granules may appear as crystals, and nylon, cotton, or vegetable fibers may be mistaken for casts or mucous threads. Such artificial structures in urine are called *artifacts*.

Summary

Collection and preparation of urine specimens for urinalysis are described. Both macroscopic and microscopic examination is outlined. Use of the refractometer and the urinometer is presented. Drawings show cellular structures, casts, and crystals found in both acid and alkaline urine.

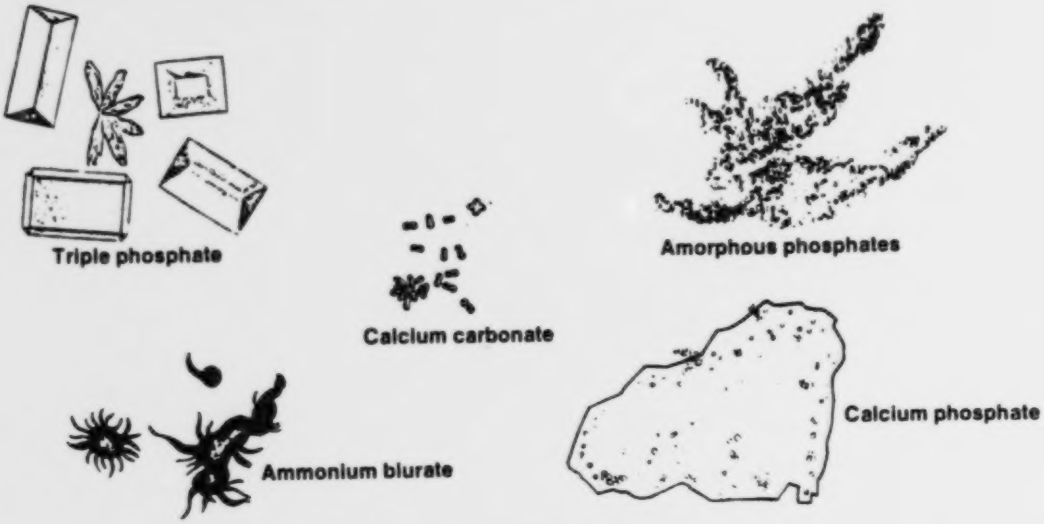


Fig. 11. Crystals in alkaline urine

6

Hematology

Objectives: After completing this unit, you will be able to—

1. *name the components of a CBC;*
2. *explain the procedure to perform cell counts using automation;*
3. *explain how to set up hemoglobin and hematocrit determinations;*
4. *calculate indices;*
5. *set up an erythrocyte sedimentation rate, and calculate corrections when needed;*
6. *explain how to perform a reticulocyte count;*
7. *explain the sickle-cell disease test procedure;*
8. *prepare, stain, and report blood smears;*
9. *explain how to set up platelet counts manually;*
10. *explain how to collect specimens for coagulation studies;*
11. *name and explain bleeding and clotting times; and*
12. *explain the significance of the prothrombin time and the activated partial thromboplastin time.*

Terms and Definitions

agranulocytes—those WBC which have few or no cytoplasmic granules.

capillary action—rise of fluid or blood in narrow tubes.

CBC—complete blood count; that series of separate hematology tests routinely done at a particular institution.

cuvette—a small glass container used to hold liquids that are to be examined photometrically.

differential white count—report of percentage and kind of leukocytes.

ESR—erythrocyte sedimentation rate; rate at which red cells settle out in a specified time as a result of changes in the surface charge of red cells.

granulocytes—those WBCs which have distinct granules in the cytoplasm.

HbS—hemoglobin S; abnormal form of hemoglobin which causes sickling of red cells, thus altering oxygen-carrying ability.

hematocrit—measurement of the percentage of the blood that is made up by the red blood cells.

hematology—study of blood; that department which concerns itself with blood cells and components.

hemocytometer—a small glass counting chamber with a microscopic ruled area placed on the microscope stage for counting blood components.

hemoglobin—substance found in red cells which transports oxygen throughout the body.

heterozygous—condition in which genes are not identical; for example, HbS and HbA.

homozygous—condition in which genes are identical; for example, HbS and HbS.

interface—space or area that separates two materials that were previously mixed.

MCH—mean corpuscular hemoglobin; the average weight of hemoglobin in the RBC.

MCHC—mean corpuscular hemoglobin concentration; the weight of hemoglobin per volume of cells.

MCV—mean corpuscular volume; the average volume of the RBC.

MPV—mean platelet volume; the average volume of the platelet.

platelet (thrombocyte)—a small piece of cytoplasm that has broken off from a larger mass; concerned with clotting mechanism in the body.

RBC—erythrocyte or red blood cell; contains hemoglobin.

reticulocyte—young red blood cell.

WBC—white blood cell or leukocyte; serves as a defense for the body by combating bacteria and viruses.

Hematology means, literally, the study of blood. This is a reasonably good, though oversimplified, definition of the work that is performed in the hematology department. *Hematology* is the department which concerns itself with blood cells and components.

Throughout this unit, you will see the symbol µ (*mu*) used with other symbols, such as µg, µl, or µµg. This symbol µ stands for *micro*, which is a millionth part. Microgram is abbreviated µg; microliter, µl; and micromicrogram, µµg (which is 10^{-12} grams).

The Complete Blood Count

The most frequently ordered test in hematology is the *complete blood count*, or *CBC*. The CBC is routinely required on all patients admitted to a hospital. The CBC is actually a group of separate tests. Though the separate tests that make up a CBC may vary somewhat from one institution to another, a typical CBC at a hospital may consist of the following tests:

- White blood count (WBC)
- Red blood cell count (RBC)
- Hemoglobin (Hgb)
- Hematocrit (Hct)
- Red blood cell indices:
    - Mean corpuscular volume (MCV)
    - Mean corpuscular hemoglobin (MCH)
    - Mean corpuscular hemoglobin concentration (MCHC)
- Platelet count (Plt)
- Differential white cell count (diff)

Other tests frequently performed in hematology are the erythrocyte sedimentation rate (sed rate or ESR), reticulocyte count (retic count), and the sickle-cell tests.

The CBC is a general indicator of a person's state of health. Yet a person may be ill even though all the tests that make up the CBC are within normal ranges. Generally, however, one or more of the tests will be abnormal with results too low or too high. Remember, when working with a living, biological system, the word *always* rarely applies. Following are some general statements relative to some of the tests included in the CBC.

White blood cells combat bacteria when an infection is present in the body. An abnormally high WBC usually means an infection. An extremely high WBC might indicate leukemia. Low WBC, called *leukopenia*, can be caused by viruses, radiation, and drugs.

Red blood cells contain a chemical called *hemoglobin*, located inside the RBCs, that carries oxygen throughout the body. Persons who have too few red cells or too little hemoglobin inside the red cells are said to be *anemic*. There are many possible causes of anemia. It may be as serious as leukemia or as simple as not getting enough iron in the food eaten. An RBC and an Hgb alone cannot diagnose the cause of anemia. Other tests must be performed to determine the actual cause of the anemia. An *erythrocytosis* is an increased RBC. This occurs in people living at high altitudes or who have polycythemia vera, a disease.

The *hematocrit* is a measurement of the percentage of the blood that is made up by the red blood cells. For example, a hematocrit value of 36% would mean that 36% of the total blood volume consists of RBCs. A rule of thumb relationship between the hematocrit and the hemoglobin is that, numerically, the hematocrit value is usually three times the hemoglobin. For example, a hemoglobin of 11.0 grams should have a hematocrit value of 33%.

Thrombocytes or platelets are small pieces of cytoplasm that initiate the clotting process when a person is injured. When the number of platelets in the bloodstream is very low, internal bleeding may occur or the skin may develop severe bruises from only a slight impact with an object. If a person with a very low platelet count does receive a cut or laceration, it may be very difficult to stop the resulting bleeding.

The differential white cell count and blood smear evaluation can provide much valuable information. A thin smear of the blood to be examined is made on a glass slide, stained, and then observed under the microscope. The various types of WBCs seen on the smear are categorized and tallied until 100 WBCs have been counted. Each cell type counted is then reported as a percentage. For example, if 23 of the 100 WBCs counted were classified as lymphocytes, then 23% lymphocytes would be reported. The size, shape, and appearance of the red blood cells are also observed, and written comments concerning any abnormalities are included in the report. The approximate number, size, and shape of the platelets are also observed and commented upon if abnormalities are seen.

Automated Cell Counts

The procedure for performing cell counts, either RBC or WBC, will be discussed. The Coulter methodology will be described for cell count technique. In the 1980 College of American Pathologists Proficiency Survey for Hematology, 2,815 of 3,724 laboratories participating nationwide used some type of Coulter® instrument. That is about three-fourths of these laboratories. Thus, it is statistically probable that the student would encounter a Coulter® instrument. No recommendation of nor preference for Coulter® products is intended or implied by the publisher.

The blood cells suspended in an electrolytic solution can be sized and counted by passing them through an opening (aperture) through which an electric current is flowing. As the cells pass through the aperture and displace an equal volume of electrolyte, the electrical resistance in the path of the current changes. This results in corresponding current and voltage changes. The amount of this change is directly proportional to the size of the cell. As a specific volume of blood sample is drawn through the aperture by vacuum, each voltage change is counted. This count represents the number of cells in the sample.

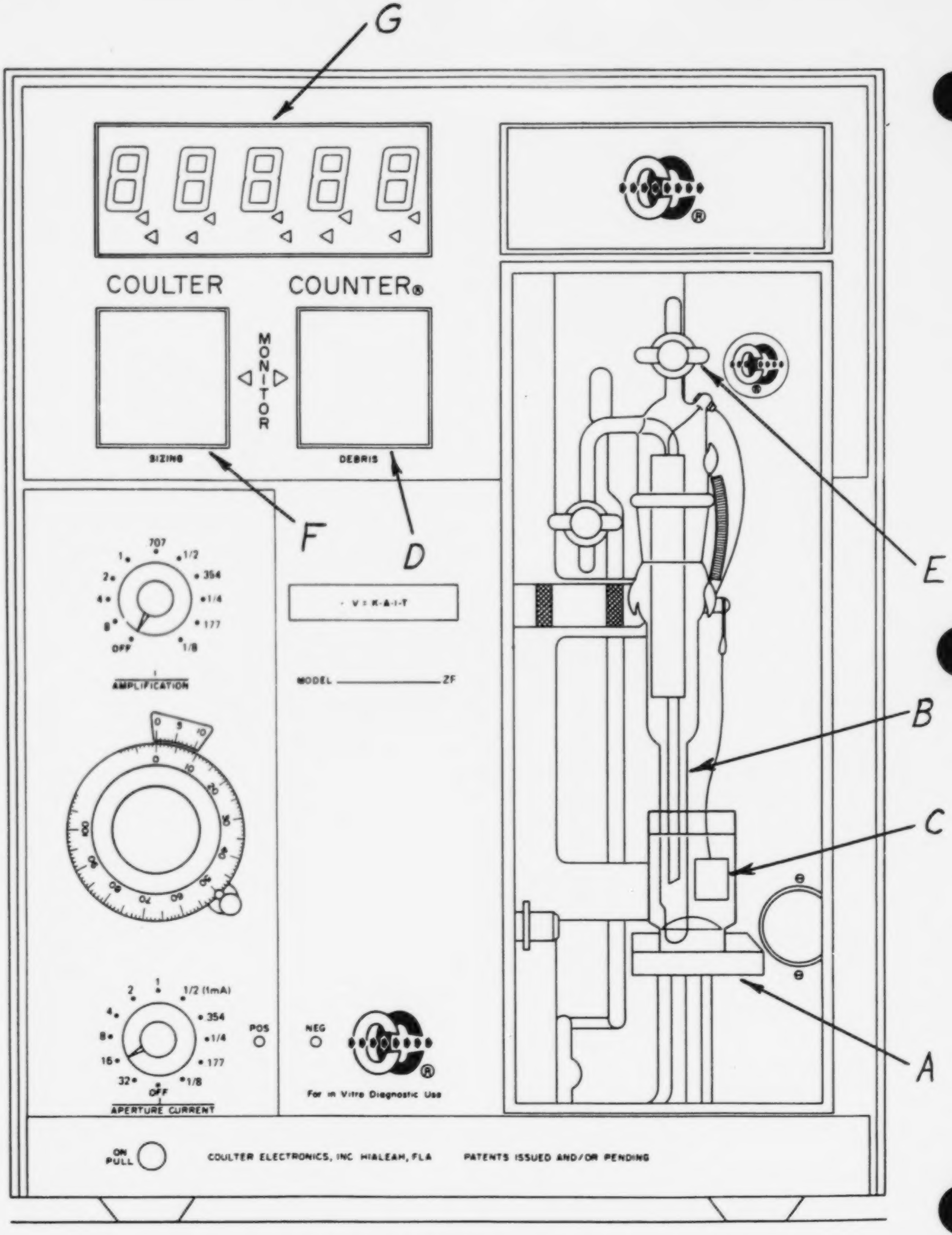
To perform a WBC and RBC count using an automated Coulter® instrument, such as the Model ZF shown in figure 1, you need the following equipment:

- Isotonic buffered saline solution (diluent)
- Two snap-cap counting vials
- 20 µl pipette
- 10 µl pipette
- 0.1 ml pipette
- Lysing reagent

Pipette 10 ml of diluent into each of the two counting vials. Into one vial, pipette 20 µl of the blood sample. Recap the vial, and mix by slowly inverting several times. The first vial contains a 1:500 dilution of the blood sample and will be used for the white cell count. The second vial contains a 1:50,000 dilution and will be used for the red cell count.

To the first vial (1:500 dilution) add three drops of lysing reagent to destroy the RBCs. Recap and invert to mix. To perform the actual counts on the Model ZF, the procedure is the same for both the red count and the white count (see fig. 1):

1. Lower the beaker platform (A), and place the vial to be counted on the platform.
2. Slowly raise the platform, immersing the aperture tube (B) and external electrode (C) into the sample.
3. Position the vial so that the aperture is clearly visible on the debris screen (D).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COULTER COUNTER®
MONITOR
SIZING
DEBRIS
F
D
E
B
C
A
707
1/2
354
1/4
177
1/8
OFF
8
4
2
1
AMPLIFICATION
V = K·A·I·T
MODEL ZF
1/2 (1mA)
354
1/4
177
1/8
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APERTURE CURRENT
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Fig. 1. Coulter Counter, Model ZF. (Reprinted by permission of Coulter Corporation)

4. Open the control stopcock (E) to a vertical position.
5. When electrical peaks appear on the sizing screen (F), close the control stopcock (E) to the horizontal position. This initiates the counting process, and the numbers of the cells being counted will appear on the digital display (G).
6. When counting stops, observe the final number on the digital display screen (G).

There is a statistical probability that two cells may pass through the aperture at exactly the same time. To account for this, Coulter provides a Coincidence Correction Chart. To correct for coincidence, find the number obtained from the digital display screen on the chart, and read the corrected number in the adjacent column. All red cell counts are to be corrected. White cell counts above 10,000 should be corrected.

When a red count is performed, the digital display result will be in millions of cells per cubic millimeter of blood. For example, a reading of 4.32 means 4.32 million RBCs per mm^3. Find 432 on the Coincidence Correction Chart, and read the corrected value (481). Thus, the value reported will be 4.81 million/mm^3.

White counts will be displayed in thousands. For example, if the display reads 6532, this means 6,532 WBCs per cubic millimeter. For practical purposes, round off the answer to the nearest 100. Thus, 6,532 becomes 6,500/mm^3. Remember to correct for coincidence on white counts above 10,000.

Manual Counts

Sometimes a manual cell count is needed. However, the technologist will perform this test with special equipment. Perhaps the supervisor at the laboratory will at some time demonstrate how to "charge" a counting chamber and will allow the student to practice WBC and platelet counts.

Hemoglobin Determination Using the Hemoglobinometer

To perform a hemoglobin determination using the Coulter® Hemoglobinometer, you will need the following supplies and equipment: counting vial, isotonic buffered saline diluent, 10 ml pipette, 20 µl pipette, and hemoglobin lysing reagent.

Follow this procedure. Pipette 20 µl of the blood to be tested into the vial. Add three drops of hemoglobin lysing reagent. Recap vial, and mix by inverting several times. Open the lid of the Hemoglobinometer, and pour the sample into the cuvette until it is completely filled. Wait 5 minutes to allow any bubbles in the specimen to rise to the top of the cuvette. Close the lid. Read the results on the digital display in grams per deciliter (gm/dl).

Cyanmethemoglobin Technique for Hemoglobin Determination

The following supplies and equipment will be needed to perform a hemoglobin using the cyanmethemoglobin technique: spectrophotometer or colorimeter, 5 ml pipette, 20 µl pipette, test tubes of greater than 5 ml capacity, optically matched cuvettes for spectrophotometer or colorimeter, Drabkin's reagent, and cyanmethemoglobin standard of known concentration.

Follow this procedure. Pipette 5 ml of Drabkin's reagent into a test tube. Pipette 20 µl of blood to be tested into the tube with Drabkin's reagent. Mix by inverting, and allow to stand 5 minutes for color development. Set the spectrophotometer at a wavelength of 540 nanometers (nm). Into a matched cuvette, pipette 5 ml of Drabkin's reagent. Place cuvette in spectrophotometer, and adjust to 0.0 optical density (O.D.). Into another matched cuvette, pipette 5 ml of standard. Place in the instrument, and read the O.D. of the solution. Pour the prepared specimen, which has been standing for 5 minutes, into a matched cuvette, and place in the instrument. Read the O.D. of this unknown specimen. Calculate the results according to the following formula:

$$\frac{\text{O.D. unknown}}{\text{O.D. standard}} \times \text{concentration of standard} = \text{hemoglobin (gm/dl)}$$

The Hematocrit

To perform a hematocrit, the following equipment will be needed: capillary tubes, sealing clay, microhematocrit centrifuge, and a microhematocrit reader.

Use this procedure. By capillary action, fill a capillary tube about three-fourths full of the blood to be tested. Seal the tube by pressing one end of the capillary tube into sealing clay. Place the sealed tube into one of the grooved slots in the head of the mcirohematocrit centrifuge. The sealed end of the tube should rest snugly against the rubber cushion at the outer edge of the centrifuge head. Screw on the cover plate securely. Close the centrifuge lid, and turn the timer on for 3 to 5 minutes as determined locally. Remove the spun tube from the centrifuge, and read on any of several commercially available types of microhematocrit readers.

RBC Indices

The RBC indices are mathematical calculations of cell size and hemoglobin content. *Mean corpuscular volume* (MCV) is the average volume of the RBC. It is obtained by dividing the product of the hematocrit (Hct) times 10 by the RBC in millions:

$$\text{MCV in } \mu^3 = \frac{\text{Hct} \times 10}{\text{RBC (in millions)}}$$

For example, if the hematocrit is 45 and the RBC is 5,000,000/mm^3, then

$$\text{MCV} = \frac{450}{5} = 90\ \mu^3$$

Mean corpuscular hemoglobin (MCH) is the average weight of hemoglobin in the RBC. It is obtained by dividing the product of the hemoglobin (Hgb) times 10 by the RBC in millions:

$$\text{MCH in } \mu\mu\text{g} = \frac{\text{Hgb} \times 10}{\text{RBC (in millions)}}$$

For example, if the hemoglobin is 15 gm/dl and the RBC is 5,000,000 mm^3, then:

$$\text{MCH} = \frac{150}{5} = 30\ \mu\mu\text{g}$$

Mean corpuscular hemoglobin concentration (MCHC) is the weight of hemoglobin per volume of cells and therefore is an indicator of the hemoglobin concentration of the average cell independent of cell size. It is calculated by dividing the hemoglobin (Hgb) by the hematocrit (Hct) and multiplying by 100:

$$\text{MCHC in } \% = \frac{\text{Hgb}}{\text{Hct}} \times 100$$

For example, if the hemoglobin is 15 gm and the hematocrit is 45%, then:

$$\text{MCHC} = \frac{15}{45} \times 100 = 33\%$$

The normal ranges for the RBC indices are these:

- MCV = 82–92 μ^3
- MCH = 27–31 $\mu\mu$g
- MCHC = 32–40%

Sedimentation Rates (ESR)

Variations in the sedimentation rate result from changes in the surface charge of red cells. These changes are related to alterations in the plasma. Increased sed rates correlate, in a very general and nonspecific way, to inflammatory processes that may be occurring in the body. Sed rates (ESR) may best be used to monitor a patient's progress or therapy. One isolated ESR does not really tell the doctor a great deal.

To perform a Wintrobe method ESR, you will need: Wintrobe sedimentation tube, transfer pipette, Wintrobe sed rate stand, timer, and sed rate correction chart.

Follow this procedure. Using a transfer pipette, fill a Wintrobe tube to the "0" mark with the blood to be tested. This is most easily done by placing the tip of the blood-filled transfer pipette all the way to the bottom of the Wintrobe tube, then allowing the blood to flow into the tube out of the pipette. As the tube fills, raise the tip of the pipette so that it is barely submerged in the rising column of blood. The filled Wintrobe tube must be free of bubbles. Place the filled tube vertically in a Wintrobe sed rate stand. Set a timer for exactly 60 minutes. Read the level in millimeters to which the red cells have settled after 1 hour. This value is the uncorrected sed rate. To correct the sed rate, use a Wintrobe chart which will be provided by the health care facility. The hematocrit value for the blood being tested must be known; so be sure to perform a hematocrit.

Newer methods of performing ESR determination used in some laboratories require only 10 minutes. The method used is determined by the preference of the lab director and the budget of the lab.

Reticulocyte Count

Reticulocytes are young red blood cells. They are distinguished by blue granules inside the cell when stained with a supravital stain such as methylene blue. This test is used to determine whether appropriate numbers of new RBCs are being produced by the bone marrow.

To perform a reticulocyte (retic) count, you will need the following supplies and equipment: freshly filtered methylene blue N solution, 12 x 75 mm test tube, dropper, glass slides, and microscope.

Follow this procedure. Using a dropper, place four drops of filtered methylene blue solution into a 12 x 75 mm test tube. Add four drops of the blood to be tested to the test tube containing the methylene blue solution. Mix by drawing the stain/blood mixture up into the dropper and expelling gently several times. Let the mixture stand for 10 minutes at room temperature. Mix again in the same manner, then prepare four thin smears on glass slides. Using an oil-immersion lens, count 1,000 RBCs. Keep a separate tally of the retics seen while counting the 1,000 RBCs. Count about 250 RBCs on each of the four slides. Divide the number of retics seen per 1,000 RBCs by 10 to determine the percentage of reticulocytes. For example, 15 retics per 1,000 RBCs equals 1.5% reticulocytes.

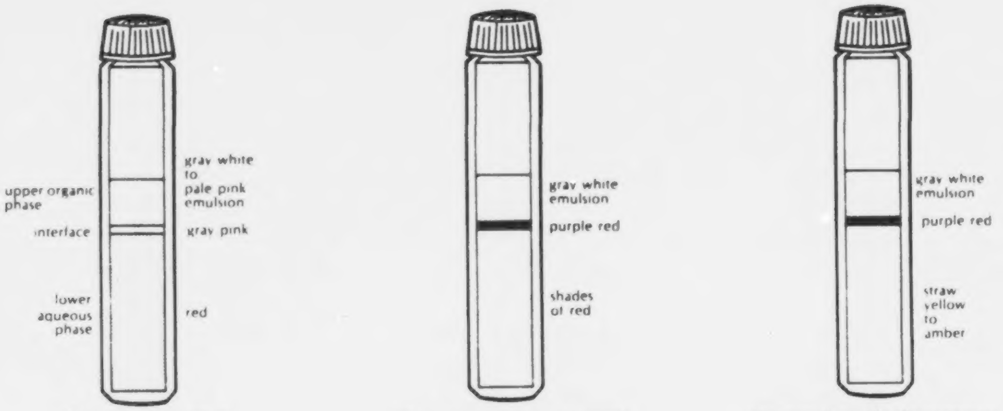
Sickle-Cell Anemia

Sickle-cell disease is an inherited disease characterized by the presence of an abnormal hemoglobin (HbS). If a gene for HbS is inherited from each parent, the person is homozygous and will have *sickle-cell anemia.* If a gene for HbS is inherited from only one parent, the person is heterozygous and will have *sickle-cell trait.* This does not usually create problems unless the person is subject to reduced oxygen tension, such as during extreme physical exertion. Figure 5 shows the abnormal sickle-shaped erythrocytes. These can obstruct capillaries, interfere with oxygen transportation, and cause pain in the joints.

To check for sickle-cell anemia using the Sicklequik method, you will need the following: three Sicklequik reaction tubes, three 0.1 ml pipettes, centrifuge, positive control (blood containing HbS), and a negative control (blood without HbS).

Label one reaction tube "patient" and the other tubes as positive and negative controls. Into the patient tube, pipette 0.1 ml of blood to be tested. Into the control tubes, pipette 0.1 ml of the blood selected for each control. Recap the tubes, and shake them vigorously for 10 seconds. Allow the tubes to stand for 5 minutes, then centrifuge all tubes for 5 minutes at 3,400 rpm. Also, this test can be performed without a centrifuge. Allow the tubes to stand for at least 2 hours (but not more than 72 hours) before interpreting the results.

If HbS is present, a dark red layer will form at the interface. The interface is the point between the upper organic phase and the lower aqueous phase. The lower aqueous phase will



Negative for HbS

Genes from one parent for HbS (Heterozygous)

Genes from both parents for HbS (Homozygous)

Fig. 2. Using the Sicklequik ® method to check for sickle-cell anemia. (Used with permission of Organon Teknika Corp.)

be varying shades of straw, yellow, or amber or shades of red. If HbS is not present, the interface will be grayish, and the lower aqueous portion will be dark red (see fig. 2).

Blood Smears and Differential Cell Count

The preparation of good blood smears is very important to anyone working in the hematology department. Some laboratories prefer slide preparations. Others prefer cover slip preparations. We will be concerned with slides. The only equipment required is two glass slides and a drop of blood.

Place a small drop of blood on a glass slide, about 1/2 inch from one end (see fig. 3). Lay the slide on a flat surface. Place the end of a second glass slide, held at an angle of about 30 degrees, on the first slide in front of the drop of blood (fig. 3A). Pull the spreader slide back into the drop of blood. When the blood has spread about two-thirds the width of the slide, push the spreader slide forward with a steady, even motion (fig. 3B). The angle of the spreader and the speed of pushing may be changed to vary the thickness. Allow the smear to air-dry. Ideally, the smear should have a "feather" edge. The feathered edge is the area chosen for counting because cells should be in a single layer without overlapping.

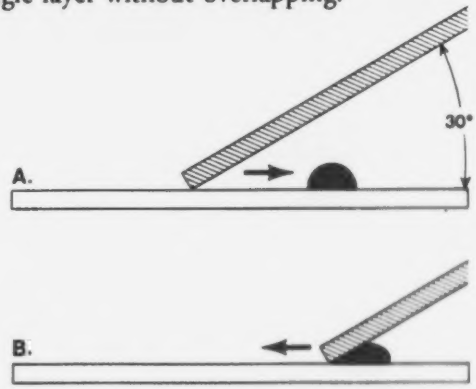


Fig. 3. Preparation of blood smears using slides

Wright's stain or some variation of Wright's stain is used to stain the blood smear. Various laboratories will have their own staining technique. They may use automatic stainers, staining jars, or staining racks. We will describe a technique using trays. Place the air-dried blood smear slide on a staining tray with the blood film facing upward. Flood the smear with Wright's stain solution. Let stand for 1 to 2 minutes. Then add an approximately equal volume of buffer solution to the slide. Blow the surface of the stain-buffer mixture to ensure proper mixing. Let stand for 3 to 5 minutes. Flood with tap water to remove the stain-buffer mixture. Wipe off the underside of the slide. Stand the slide on end in a drying rack to drain and air-dry. Instruments are available to automatically fix, stain, and dry peripheral blood smears in about 3 minutes. These instruments can also be used to stain smears of bone marrow and other body fluids.

The hematology department can give the physician much valuable information by reporting completely and accurately what is observed while doing the differential cell count (diff). After the thin smear of blood is made on a glass slide and stained, it is observed under the microscope. Place a drop of immersion oil on the stained slide, and observe on high dry for anything unusual. Switch the objective to the oil-immersion lens. The various types of WBCs seen on the smear are categorized and tallied until 100 WBCs have been classified. Each cell type counted is then reported as a percentage. For example, if 23 of the 100 WBCs counted were classified as lymphocytes, then 23% lymphocytes would be reported.

Leukocytes are those blood cells which take up the purple-blue-pink-red stain. All average 9–13 μm in size. Leukocytes (WBC) are of two general varieties: *granular*, those which have distinct granules in the cytoplasm, and *agranular*, those which have few or no cytoplasmic granules.

The granular leukocytes (*granulocytes*) can be distinguished from each other by the color of their granules. *Eosinophils* (eos) have distinct red cytoplasmic granules (see fig. 4). *Basophils* (basos) have large blue cytoplasmic granules. *Neutrophils* have a light pink cytoplasm with small, numerous, evenly distributed light pink to bluish black granules. As the granular leukocytes mature, the nucleus tends to become separated into distinct lobes (which take up the dark stain) with a narrow filament connecting the lobes. These are called *polys* or *segs*. A *band* is a neutrophil whose nucleus has lengthened but has not separated. A *juvenile* has a slightly indented nucleus.

The agranular leukocytes are the lymphocytes and monocytes. The *lymphocytes* (lymphs) have blue cytoplasm, nonsegmented nuclei, and are 7–15 μm in size. They may or may not have granules. The *monocytes* (monos) have variable shapes and a dull gray blue cytoplasm with numerous, fine, lightly stained, evenly distributed granules. The nucleus has brainlike convolutions and may be linear, E-shaped, or horseshoe-shaped. Monocytes are the largest leukocyte at 12 to 20 μm.

The technologist not only reports the percentage of each kind of WBC, but also looks at the size and shape of the red blood cells. Normally, erythrocytes stain a pinkish tan, are 7–8 microns in size, and have no nucleus. If they vary in size or shape, such terms as macrocytic (larger than normal), microcytic (smaller than normal), poikilocytosis (various shapes), anisocytosis (all sizes on the smear), and polychromatophilic (variation in color) are used to describe the RBCs. Figure 5 shows various RBCs that might be seen on a smear. The crenated forms occur when the red blood cells begin to shrink due to water loss. The target cells stain darker in the center and around the edges, like a target. These occur in certain types of anemia.

The approximate number, size, and shape of the platelets are also observed and commented upon if abnormalities are seen. Platelets appear as small bluish round or oval fragments measuring about 2–3 μm in diameter.

Platelet Counts

Platelet counts may be performed using many different techniques. If the laboratory is fully automated, the platelet count is done along with the determinations for WBC, RBC, hemoglobin, and hematocrit. In addition to a count of the number of platelets present, automated hematology departments provide MPV on their printouts. MPV is the *mean platelet volume* or the average volume of the platelet.

The Unopette method will be discussed here. You will need the following equipment: Unopette reservoir containing diluent for

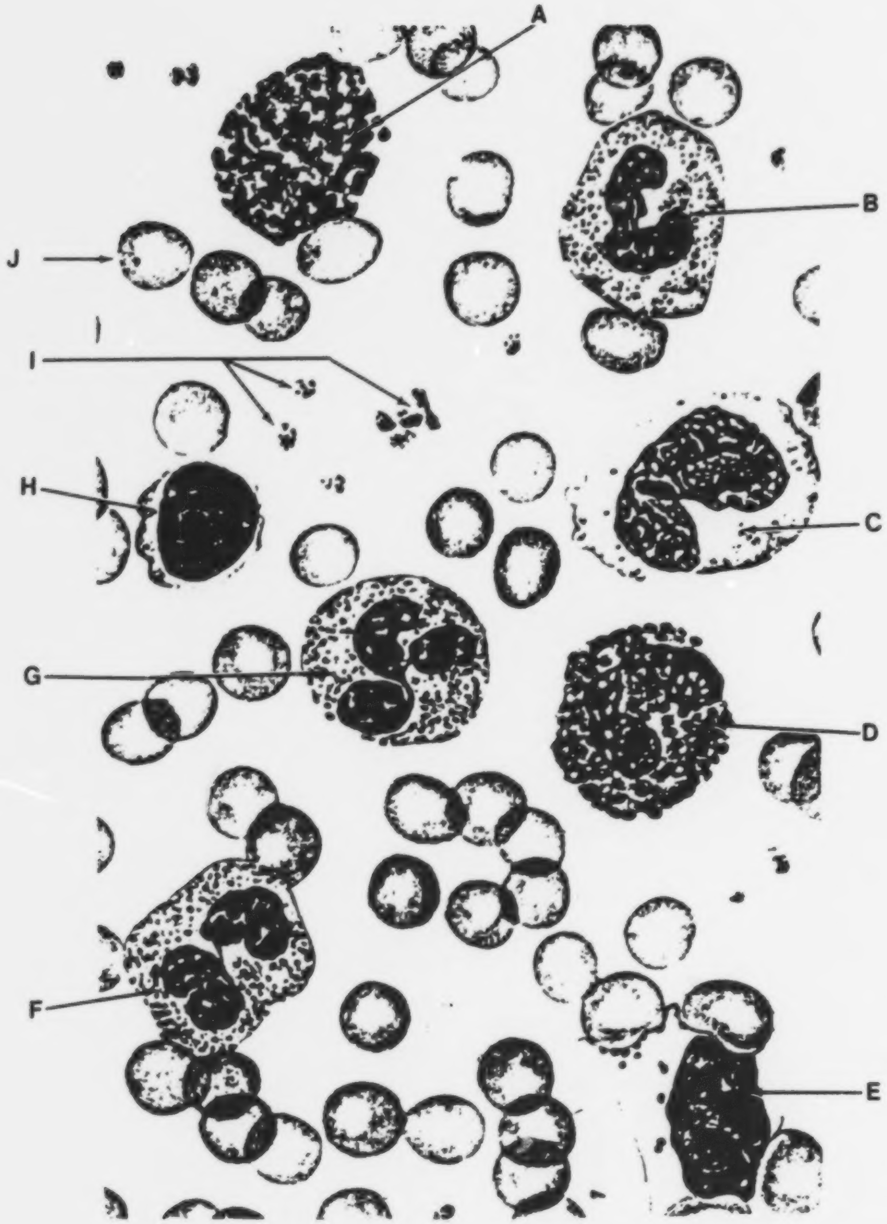


Fig. 4. Types of blood cells stained with Wright's stain. Basophil (A), band (B), monocyte (C), eosinophil (D), lymphocytes (E and H), neutrophils or segs or polys (F and G), platelets (I), and RBC (J)

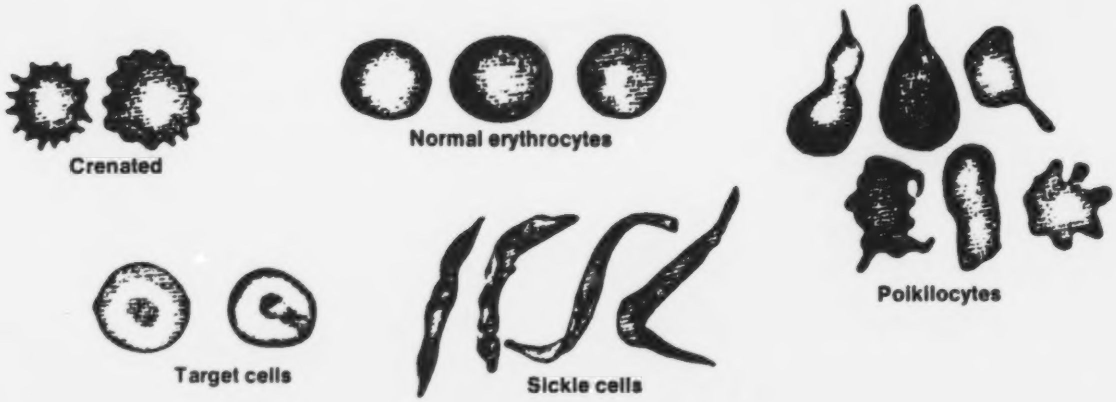


Fig. 5. Various RBCs that might be seen on a smear

platelet determination, Unopette capillary pipette (20 µl), microscope, counting chamber (fig. 6), petri dish, and counter.

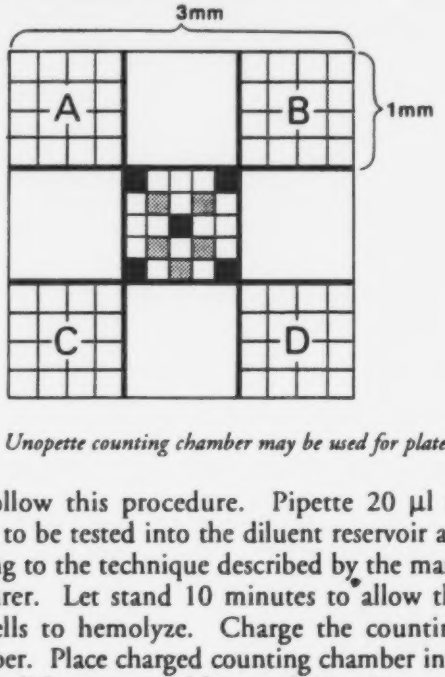


Fig. 6. Unopette counting chamber may be used for platelet counts

Follow this procedure. Pipette 20 µl of blood to be tested into the diluent reservoir according to the technique described by the manufacturer. Let stand 10 minutes to allow the red cells to hemolyze. Charge the counting chamber. Place charged counting chamber into a petri dish, cover, and let stand 10 minutes to allow cells to settle. The bottom of the petri dish should be covered with moistened filter paper to retard evaporation of the specimen. Remove the counting chamber from the petri dish, and place it on the microscope stage. Locate the area to be counted on low power. Then switch to the high-power objective, and count the platelets in all 25 small squares within the large center square. Multiply the number of platelets counted by 1,000 to get the total platelet count. If 250 platelets were counted, the total count would be:

$$250 \times 1{,}000 = 250{,}000 \text{ platelets/mm}^3.$$

Preparing for Coagulation Studies

The collection and handling of specimens for coagulation (coag) tests is very important. By means of a patented system, blood is aspirated from the vein directly into evacuated glass tubes containing specified amounts of anticoagulant. These precalibrated tubes draw a specified amount of blood to yield the proper 9:1 ratio of blood to anticoagulant. For prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen essay, factor identification, factor assay, fibrin split products, and any additional tests on plasma, 4.5 ml of whole blood is added to 0.5 ml of 3.8% sodium citrate.

After proper identification of the patient, a clean (quick and accurate) venipuncture is necessary with minimal tourniquet pressure preferred. Blue-top (citrate) tubes for the coag test should be collected after first drawing the

EDTA (lavender-top) tubes for cell counts and the red-top (no anticoagulant) tubes for chemistry and serology. The top colors refer to Becton Dickinson VACUTAINER tubes.

The first tube is most likely to be contaminated with small amounts of tissue thromboplastin, which can activate the extrinsic coagulation pathway and lead to variable coag results. Prompt and proper mixing of all blue-top tubes must be done to bind all the calcium immediately.

The volume of blood aspirated must be correct for tube size and amount of anticoagulant. Comparison tubes, calibrated and marked to show proper filling, should be available near the centrifuge. Tubes must also be checked for clots and hemolysis.

The tests to be performed will determine the time and speed of centrifugation. When platelet-poor plasma is desired for PTs, APTTs, fibrinogen assays, etc., spin the capped tubes at full speed of 2,000+ rpm for 10 minutes. When multiple tests are to be performed and platelet-poor plasma is desired, a disposable serum-plasma separator device, such as Sure-Sep, may be inserted before centrifugation. Precaution should be exercised to avoid skin contact with the blood when inserting a separator device.

Since coagulation tests are enzymatic procedures, reaction temperature must be controlled at 37°C by instrumentation. Other variables in coagulation studies are the choice of anticoagulant (citrate is preferred rather than oxalate) and loss of CO_2 and rising pH (due to unstoppered vs. stoppered tubes). If the specimen cannot be immediately tested, parafilm-sealed tubes may be stored in the refrigerator for 1 to 4 hours. If testing will be delayed longer than 4 hours, plasma should be placed into stoppered tubes and frozen.

Bleeding Time

The bleeding time is that time between the making of a small incision and the moment bleeding stops. It is a test of platelet function and is frequently used as a presurgical screening test. Many methods have been developed for performing this test. Among them are the Ivy, the template, and the Duke, or modifications of these. The original bleeding time that Duke described was performed by making puncture wounds on the forearm while a constant pressure of 40 mm was held on that arm by using a sphygmomanometer. The modified Duke method uses the lobe of the ear. The lobe is cleaned, allowed to dry, pierced with a firm stroke, and allowed to bleed onto filter paper. A stopwatch is started. Blood is removed with the filter paper every 30 seconds without touching the puncture until the bleeding stops. This time is recorded. If bleeding has not stopped within 10 minutes (Duke) and 15 minutes (Ivy), results are abnormal. Testing is stopped and pressure applied to the wound to stop bleeding.

Clotting Time

Methods for performing clotting times also vary from laboratory to laboratory. The capillary clotting time is widely used. The capillary method uses blood from the finger, toe, and earlobe placed in three capillary tubes. It is important to use a rapid, free flow of blood that has not been obtained by squeezing or manipulation of the puncture wound. Timing begins with the appearance of the second drop of blood and continues to the formation of a fibrin thread as a capillary tube is broken.

Coagulation of Blood

Coagulation consists of changing fibrinogen (one of the plasma proteins) into fibrin by the use of thrombin and calcium salts. Fibrinogen, calcium salts, prothrombin, and antiprothrombin are normal, constant constituents of the plasma. Prothrombin and antiprothrombin are held in inactive combination, so coagulation within the vessels does not occur. Thromboplastin, which accelerates coagulation, is not present in the circulating blood but is located in the tissue fluid. When blood escapes from vessels, it comes in contact with the thromboplastin in the tissues. This neutralizes the antiprothrombin, which sets the prothrombin free to react with the calcium salts. The prothrombin is transformed into thrombin. This, in turn, transforms fibrinogen into fibrin, which then forms a meshwork of threads to entangle the blood cells. It is thought that disintegrating platelets contain enough thromboplastin to liberate the antiprothrombin and start the coagulation process.

thromboplastin + calcium salts + prothrombin→thrombin
thrombin + fibrinogen→fibrin

Blood for prothrombin time and partial thromboplastin time must be collected in a test tube containing a special anticoagulant. Most laboratories use a 9:1 ratio of blood to 3.8% sodium citrate. Other anticoagulants used are sodium oxalate and EDTA (ethylenediamine tetra acetic acid). A 3.8% solution of sodium citrate may be obtained prepackaged within VACUTAINERS in sealed cans from Becton Dickinson VACUTAINER Systems, Rutherford, New York. They are stored at room temperature and are stable for a year.

Prothrombin Time

The prothrombin time is also referred to as PT, protime, prothrombin time test, or a PT test. A ***prothrombin time*** is a measure of the clotting ability of a series of combined coagulation factors in plasma. Prothrombin is a protein substance produced by the liver and required for the clotting of blood. Although the prothrombin time does not directly measure the amount of prothrombin, the test is a suitable clinical means of determining the presence and functioning ability of prothrombin.

Patient plasma is added to a mixture of calcium and thromboplastin to form a clot. The time interval between the addition of the plasma to the calcium-thromboplastin mixture and the appearance of the clot is called the prothrombin time. Blood is collected in a 9:1 ratio of blood to sodium citrate. These are thoroughly mixed so that the calcium in the blood binds with the anticoagulant and is thus removed from the plasma. Since calcium is one of the factors necessary in coagulation, it must be restored to the system during the test procedure.

The function of the thromboplastin in the protime test is to assist the prothrombin in converting to thrombin so a clot can form. Normal values for a protime are 10 to 16 seconds. The protime is often used in the diagnosis of disease. It is more useful during the treatment of blood clots. When a patient has a blood clot, he/she receives a series of treatments with an anticoagulant, such as dicumarol or heparin. These drugs delay coagulation—preventing existing blood clots from expanding and new clots from forming. Since the anticoagulant delays clotting, it causes the protime results to be above normal. This necessitates the monitoring of the patient's clotting time (therapeutic range is 28 to 40 seconds). If the protime rises above 40 seconds, the patient could hemorrhage. If the protime falls below 28 seconds, the treatment may be ineffective, causing old clots to expand or new clots to form. The protime may be performed by the following methods. The manual method is the tilt tube method or wire loop method. Automated methods are Fibro System, Electra 750, Hemochron, or Data-fi. No matter which method is used, the protime is a valuable test and must be performed with extreme care.

The activated partial thromboplastin time (APTT) is the single most useful procedure available for routine screening of coagulation disorders. The APTT measures the coagulation factors present. Calcium in whole blood is bound by the anticoagulant as in the protime. The plasma, after centrifugation, contains all the intrinsic coagulation factors (except calcium and platelets). Calcium, partial thromboplastin (a platelet substitute), and kaolin are added to the plasma. The time required for the plasma to clot is the APTT. Normal range for an APTT is 22.5 to 32.5 seconds by the Electra 750 method. When the APTT is longer than normal, this may be due to a deficiency in one of the coagulation factors, or it may be due to anticoagulation factors, or it may be due to anticoagulants circulating in the patient's plasma.

Summary

Hematology means the study of blood. Many tests have been devised to assay the various components and properties of blood. The complete blood count has the widest usage. The CBC includes tests to determine the number and kind of leukocytes and erythrocytes and the hemoglobin content. These tests may be done by automation or manually. Sedimentation rate, reticulotyte count, Sicklequik, and platelet counts are done to determine abnormal conditions. Slides for differential cell counts require special preparation. Coagulation studies are also done in hematology. Some of the tests used to determine problems with coagulation are bleeding time, coagulation time, prothrombin time, and the activated partial thromboplastin time.

7

Serology

Objectives: After completing this unit, you will be able to—

1. *name and perform tests for syphilis, such as the RPR;*
2. *perform and/or identify other serological tests to detect—*
   - *a. C-reactive protein,*
   - *b. rheumatoid arthritis,*
   - *c. infectious mononucleosis,*
   - *d. febrile agglutinations,*
   - *e. ASO titers, and*
   - *f. cold agglutinins;*
3. *perform pregnancy test on urine or serum; and*
4. *describe tests used to detect AIDS.*

Terms and Definitions

agglutination—the collection into clumps of the cells that are distributed in a fluid.

agglutinin—specific antigen used in agglutination tests.

AIDS—acquired immunodeficiency syndrome.

antibody—proteins developed within the body in response to a foreign substance (antigen), often providing immunity to disease.

antigen—substance that induces the formation of antibodies.

ARC—AIDS-related complex.

atypical—irregular; not conforming to type.

buffy coat—light-colored layer of blood between the plasma and RBC; contains mostly WBC.

ectopic pregnancy—implantation of fetus outside uterine cavity.

ELISA—enzyme-linked immunosorbent assay.

febrile—pertaining to a fever.

flocculation—colloid phenomenon in which the disperse phase separates in discrete, usually visible particles rather than in a continuous mass.

fluorescent—luminous when exposed to other rays.

FUO—abbreviation for fever of unknown origin.

heterophil antibody test—test for infectious mononucleosis using a series of diluted patient sera and washed sheep red blood cells.

HIV—human immunodeficiency virus.

human chorionic gonadotropin (HCG)—hormone excreted in the urine of pregnant women.

hydatiform mole—mole formed by rapid multiplication of chorionic villi to form a mass of cysts resembling a bunch of grapes; causes false positive pregnancy test.

inactivation—to render inert by use of heat; to destroy biological activities by heat.

in vitro—that which can be observed or demonstrated in a test tube as opposed to *in vivo*, which occurs within the living organism.

lysis—destruction of cells by a specific lysin.

opsonin—substance in blood serum that acts upon microorganisms and other cells, facilitating phagocytosis.

precipitation—test reaction during which a deposit falls to the bottom, thus separating out of solution.

rickettsiae—minute rod-shaped or coccobacillary microorganisms that are parasites and cause disease in vertebrates; intermediate between bacteria and viruses.

serology—study of antigen-antibody reactions in vitro.

serum sickness—condition which develops following administration of antisera or drug therapy.

spirochete—slender, spiral, motile microorganism.

titer—highest dilution of serum causing agglutination or strength of serum.

Serology is the study of antigen-antibody reactions in vitro. *In vitro* means that which can be observed in a test tube or on a glass slide as opposed to *in vivo*, which occurs within the living organism.

Serodiagnosis

When a patient has an infectious disease, certain substances, called *antibodies*, are formed in the tissues that have the ability to destroy or affect the disease-producing agent or *antigen*. These substances are found in the blood, plasma, and lymph and appear in the serum after the blood coagulates. Generally, these "antibodies" or "immune bodies" are *specific*, and the demonstration of them indicates the presence of an infectious disease caused by a specific antigen. The antibody is often easier to detect than the disease-producing agent itself. The antibodies may appear immediately after the onset of the disease, or detection may be delayed until the disease is advanced. The antibodies remain for a variable time after recovery from the infectious disease.

Nonspecific antibodies are antibodies that are formed in response to an agent other than the bacterium that causes a given disease. For example, antibodies developed in response to tissue damage in syphilis are nonspecific.

The presence of specific antibodies may be detected by tests that demonstrate one of the following results: agglutination, flocculation, bacteriolysis, precipitation, complement-fixation, hemolysis, or the presence of opsonins.

Initially, a serology test indicated a test for syphilis. Now the area has been expanded to include tests for other pathological conditions characterized by a rise in antibodies.

Syphilis

Syphilis is caused by the spirochete *Treponema pallidum*. In response to this disease process, two antibodies develop. The treponemal antibodies are specific and are developed against the spirochete. The nonspecific antibodies are developed in response to tissue damage by spirochetes and are called nontreponemal antibodies.

The three most commonly used tests for syphilis are the (1) FTA-ABS (Fluorescent Treponemal Antibody-Absorption Test), (2) the VDRL (Venereal Disease Research Laboratory), and (3) the RPR (Rapid Plasma Reagin). The FTA-ABS test is specific and is performed to confirm reactive sera. This test is performed in large laboratories, reference laboratories, and state facilities. The VDRL test is nonspecific. It requires inactivated serum and can be done easily in most laboratories. The RPR test is also nonspecific. It can be performed on plasma and is more sensitive but less specific than the VDRL. The RPR does not require inactivation of serum and can be done easily and rapidly in any laboratory. Less frequently used tests for syphilis are the Wasserman, Kahn, colloidal gold, TPI (Treponema Pallidum Immo-bilization), Plasmacrit, and dark field microscopy.

Procedure for RPR Test

To perform the RPR test, follow these steps:

1. Place a drop of plasma or serum onto the circle on the RPR card using a capillary tube and rubber bulb (fig. 1A).
2. Spread the drop thoughout the circle with the stirrer (fig. 1B).
3. Treat the control sera the same as the patient serum.
4. Add one drop of well-shaken antigen from the antigen dispensing bottle (fig. 1C).

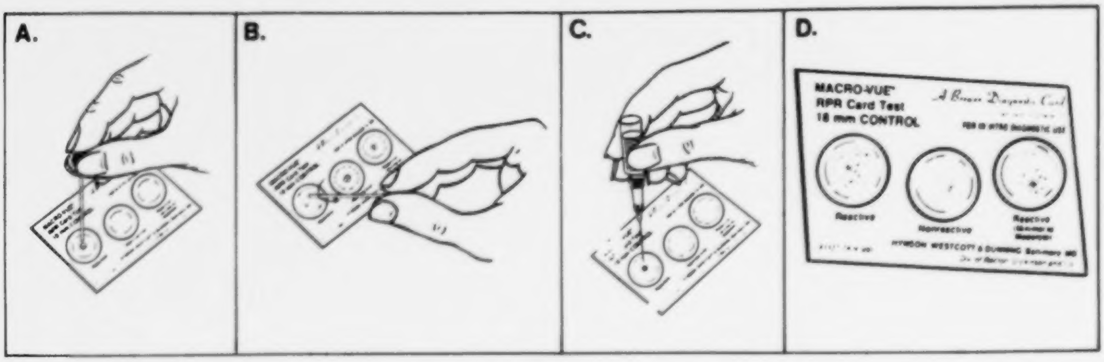


Fig. 1. Procedure for RPR test

5. Place on the mechanical rotor, and cover with humidifying cover.
6. Rotate 8 minutes.
7. Read macroscopically, and report as reactive or nonreactive (fig. 1D). Report as reactive from large clumps visible to the naked eye to small clumps that are weakly reactive. Nonreactive means no clumping.

C-Reactive Protein Test

C-Reactive protein is an abnormal protein found in persons with infectious and inflammatory conditions. The CRP test is nonspecific. The abnormal protein appears within 12 to 24 hours after the onset of the condition. It subsides when the inflammation disappears. A positive CRP may be present during the following conditions: pneumococcal pneumonia, streptococcal infections, rheumatic fever, lupus erythematosus, myocardial infarctions, and some kinds of carcinoma. It may also be present during sinusitis, pregnancy, use of oral contraceptives, and in women with an IUD in place. It is sometimes present in normal persons in the older age group.

Rheumatoid Arthritis Tests

The serum of patients with rheumatoid arthritis may contain a macroglobin (protein) antibody called rheumatoid factor (RF). This RF may be detected by its ability to agglutinate erythrocytes or latex particles during rapid slide testing. This is a nonspecific test that may also be positive in the following: lupus erythematosus, bacterial endocarditis, cirrhosis of the liver, viral hepatitis, and some forms of leukemia. Many different commercial kits are available for the detection of rheumatoid factor. You should follow the manufacturer's directions carefully. Additional testing, such as serial dilutions or ANA (antinuclear antibodies), may be required to aid in differentiating specific diseases.

Infectious Mononucleosis Tests

Infectious mononucleosis is a disease characterized by fever and an enlargement of the lymph nodes and spleen. Atypical lymphocytes and heterophil antibodies are found in the blood. In the laboratory, the technologist will distinguish the atypical lymphocytes from the normal lymphocytes and monocytes, and record the percentage of atypical cells as the differential cell count is done.

Heterophil antibodies differ from other antibodies in that they are nonspecific. For example, these antibodies in human blood serum may react with the red blood cells of sheep, causing them to agglutinate.

There are various types of tests for heterophil antibodies. A tube test consists of a series of dilutions of the patient's serum mixed in test tubes and incubated with washed sheep cells. Agglutination of the sheep cells indicates the presence of heterophil antibodies. The greatest dilution that reacts is reported.

This is a nonspecific test and therefore *presumptive.* The increase in serum agglutinins may be either from infectious mononucleosis or from serum sickness. Further tests that are specific for infectious mononucleosis should be done for differentiation.

The spot (*Mono-Test,* from Wampole Laboratories) is an example of a modified rapid differential test for infectious mononucleosis. It uses a slide instead of test tubes. The following materials and procedure may be used to perform the Mono-Test.

You will need these *materials*: centrifuge, control sera, calibrated capillary tubes and bulbs, glass slide or disposable card slide, stirrers, and Mono-Test Reagent.

Follow this procedure:

1. Centrifuge the patient's specimen. Do not use heparin during collection of the blood.
2. Fill capillary tube up to the mark with patient's serum.
3. Empty contents of tube into center of the middle section of the slide.
4. Place one drop of positive control serum on the left section of the slide.
5. Place one drop of negative control serum on the right section of the slide.
6. Add one drop of Mono-Test Reagent to each section of the slide.
7. Using a separate stirrer for each section, mix serum and reagent.
8. Spread over an area approximately 1 inch in diameter.
9. Observe for agglutination.

Use a high-intensity lamp for lateral illumination, and observe against a dark background. If using disposable cards:

1. Rock gently for 1 minute.
2. Leave undisturbed for another minute.
3. Observe immediately for agglutination.

Report as positive if agglutination of the Mono-Test Reagent occurs. Report as negative if there is no agglutination, or if the agglutination is of a finely granular pattern.

Febrile Agglutinations Tests

When a patient continues to exhibit fever for unknown reasons, a battery of tests known as *febrile agglutinations* may be ordered to determine the cause of the fever of unknown origin (FUO). This test battery is used to differentiate among typhoid fever, paratyphoid fever, tularemia, undulant fever (brucellosis), and the rickettsial diseases—typhus fever, trench fever, and Rocky Mountain spotted fever. The test detects antibodies in the patient's serum. The antigens used in testing are prepared from the specific bacteria that cause the various diseases. For example, the antigen used to test for typhoid fever is prepared from *B. typhi O* or *B. typhi H. B. paratyphoid (A, B,* or *C*) is used to test for paratyphoid fever. *F. tularensis* is used to test for tularemia, and *Brucella* is used to test for brucellosis. A nonspecific antigen using *Proteus OX-19, OX-K,* and *OS-2* is used to test for the rickettsial diseases. These antigens are available commercially.

The procedure for febrile agglutinations includes the following materials: pipettes, to deliver serum; febrile-type, glass-ringed slides with five rings across and six rings down; centrifuge; stirrers; commercial antigens; and control sera.

Follow these steps:

1. Centrifuge blood to separate serum from cells.
2. Using special febrile pipette, deliver 0.08, 0.04, 0.02, 0.01, and 0.005 ml of serum to the top row of rings across the slide.
3. Repeat procedure to the second row, and each subsequent row, until all six rows have serum (see fig. 2).
4. Be certain to prepare control serum in the same amount.
5. Add a drop of the commercially prepared antigen to each amount of serum all the way across the slide. Start at the right side of the slide.
6. Use the stirrer to thoroughly mix each ring.

7. Use a different antigen for each cross row.
8. When all rings are mixed, rotate the slide 20 times by hand.
9. Read immediately for agglutination.
10. Agglutinations of 2++ are reported.
11. Dilutions for the rings are 1:10, 1:20, 1:40, 1:80, and 1:160. If the last ring is positive (1:160), you must make further dilutions and repeat the procedure.

When testing with the *F. tularensis* antigen, place the slide on a mechanical rotator at 150 rpm for 4 minutes. Read immediately.

Your laboratory may prefer to check only the 1:10 dilutions (0.08 ml serum) before setting up the entire slide. One reactive test may not be significant and should be repeated. A rising titer 3 to 5 days apart is significant.

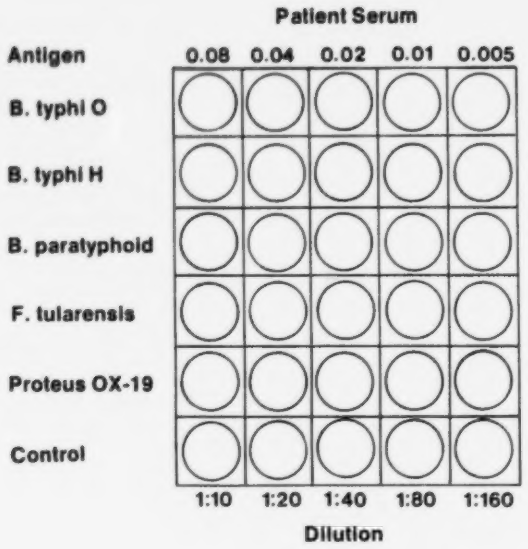


Fig. 2. Testing for febrile agglutinations using glass ringed slide

Antistreptolysin O (ASO) Test

In 90 percent of streptococcal infections, the body produces specific antibodies in response to extracellular products (Streptolysin O antigens) of Group A Streptococcus. In the body, Streptolysin O antigen produces lysis of red cells (e.g., flushed skin color in scarlet fever). Titers begin to rise about 7 days after the onset of the infection and reach maximal levels after 4 to 6 weeks.

Since there are several modifications of the ASO test, you should follow the manufacturer's instructions for the test used in your laboratory. An elevated ASO titer is helpful in diagnosis of recent Group A Streptococci infection, acute rheumatic fever, and acute glomerular nephritis.

Antinuclear Antibody Test (ANA)

Antinuclear antibodies are produced in serum in response to the nuclei of white blood cells. The test is used to discover the presence of factors associated with autoimmune diseases where the body actually attacks itself. Damage to such organs as the heart, kidneys, and liver can result. Systemic lupus erythematosus (SLE) has a certain antibody which can be detected by this test.

Cold Agglutinins Test

Many normal people have cold agglutinins in the blood. These are agglutinins that will cause the person's own red blood cells to agglutinate at temperatures between 0° and 5°C. However, even if cold agglutinins are present, they are usually demonstrable only in undiluted or slightly diluted serum. The agglutination is reversible. It breaks up on warming and occurs again upon chilling. The agglutinin can be absorbed onto red blood cells in cold temperature, thus removing them from the serum. This procedure must often be done in blood banking to achieve a compatible cross match. However, cold agglutination is of most clinical significance in confirming the diagnosis of viral (atypical) pneumonia.

The following procedure may be used to test for cold agglutinins:

1. Collect a tube of venous blood.
2. Immediately place blood in 37°C water bath for 1 hour.
3. Allow to clot undisturbed during incubation.

4. Centrifuge and remove serum.
5. Prepare mixture of red blood cells.
6. Wash three times in physiological saline.
7. Prepare 2% suspension of washed red cells (0.2 ml of cells diluted to 10 ml with saline).
8. Make serial dilutions of patient's serum.
    a. Place 0.2 ml of saline in tubes labeled #1 through #10.
    b. Add 0.2 ml of serum to tube #1, and mix thoroughly.
    c. Transfer 0.2 ml of contents of tube #1 to tube # 2. Mix thoroughly.
    d. Transfer 0.2 ml of contents of tube #2 to tube #3, and continue through tube #9.
    e. Discard 0.2 ml from tube #9.
    f. Tube #10 is the control and contains only saline.
9. Final dilutions of serum are 1:2, 1:4, etc.
10. Add 0.2 ml of 2% cell suspension to each tube.
11. Shake rack to mix.
12. Refrigerate overnight.
13. Read agglutination of 1+ or more, and report as the reciprocal of the highest dilution that shows agglutination.
14. Reversibility is demonstrated by placing in a 37°C water bath for an hour.

Pregnancy Tests

Pregnancy tests are often performed on urine specimens. They may also be performed on serum specimens. These are either test tube tests or slide tests. The results of the test tube tests are available usually in 2 hours. The results are available within minutes when a slide is used.

Most pregnancy screening tests are qualitative, and many types are now commercially available. These are easy to perform and may be purchased over-the-counter and run by the patient at home. Most of these tests measure the level of human chorionic gonadotropin (HCG) hormone excreted in the urine. The HCG hormone will be found in urine about one week after the first missed menstrual period.

For testing in the laboratory, the patient should be instructed to collect the first morning specimen in a clean container. The hormone is more concentrated at that time. These conditions may cause interference in a pregnancy test: the presence of detergent residue in the collection container, medications taken by the patient, certain pathological conditions, menopause, or ectopic pregnancy.

Some tests require filtered urine. If the sample is turbid, centrifugation may be required with testing performed on the supernatant. Some tests show hemagglutination while others demonstrate precipitation of latex particles in test tubes. Adequate controls must be prepared according to manufacturer's directions. The proper time period must be observed. A negative test result may occur in cases of very early pregnancy or if there is a low concentration of HCG. Negative tests should be repeated in 7 to 10 days as specified by the manufacturer. Quantitative tests may be performed on 24-hour specimens to determine any of these conditions: (1) threatened abortion, (2) hydatiform mole, (3) ectopic pregnancy, or (4) fetal death.

AIDS Testing

The demonstration of antibodies to HIV (human immunodeficiency virus) is used to diagnose AIDS (acquired immunodeficiency syndrome) and ARC (AIDS-related complex). Two types of tests are used: the ELISA (enzyme-linked immunosorbent assay) and the Western blot test. The ELISA is used for screening. A positive ELISA can be confirmed with the more specific Western blot test because false positive ELISA tests do occur.

For ELISA testing, patient serum is placed in a microtiter plate well or exposed to beads coated with inactivated HIV virus antigens and immunoglobulin linked with an enzyme. After inoculation and incubation, a color change occurs.

The Western blot test identifies proteins of a specific molecular weight—in this case, HIV. This test is an immunoelectrophoretic procedure.

The transmission of AIDS is believed to occur by four routes. These are listed as follows with the highest incidence first: unprotected sexual intercourse with an HIV-infected partner, sharing a needle contaminated with the virus, infection transmitted from mother to baby during gestation or birthing, and transfusion of contaminated blood or blood products. According to information from the CDC, HIV has been isolated from blood, cerebrospinal fluid, saliva, semen, vaginal secretions, breast milk, and amniotic fluid. The HIV virus has not been isolated from other body fluids, such as nasal secretions, sputum, sweat, tears, feces, urine, or vomitus unless the body fluid was contaminated with blood.

Because of the question of confidentiality in the identification of persons found to test positive for the antibodies associated with HIV, most laboratories use a strict system of identification by number only. Actual patient names are restricted to a limited number of people.

Summary

Serodiagnosis is discussed as it relates to the study of antigen-antibody reactions in vitro. The following serologic tests are outlined and/or discussed: RPR, C-Reactive Protein, rheumatoid arthritis tests, infectious mononucleosis tests, febrile agglutinations, ASO tests, antinuclear antibody test, and tests for cold agglutinins. Types of pregnancy tests are explained. Two types of tests are explained for AIDS.

8

Microbiology

Objectives: After completing this unit, you will be able to—

1. *differentiate between normal body flora and pathogens;*
2. *describe the procedure for collection of specimens;*
3. *list different types of media available for use;*
4. *inoculate, streak, and store cultures properly;*
5. *explain the use of CO_2 atmosphere;*
6. *describe the shape of bacteria and fungi;*
7. *apply antibiotic susceptibility discs;*
8. *perform a Gram stain;*
9. *perform acid-fast stain; and*
10. *collect skin scrapings for fungus study and staining.*

Terms and Definitions

aerobic—grow in the presence of free oxygen.

agar—substance used in culture media; liquid when warmed, solid at room temperature.

anaerobic—grow only if there is no oxygen.

aseptic —free of microorganisms.

bacteriology—the study of bacteria.

broth—a liquid media for growing microorganisms.

contaminant—foreign organism developing accidentally in a culture.

culture—growing microorganisms on artificial media.

dermatophyte—fungus parasite on the skin.

fix—to fasten or hold firm and not wash off with water or staining solution.

hypha—a single filament or thread of a mycelium; plural is *hyphae.*

inoculate—the introduction of microorganisms onto culture medium.

inoculating loop/needle—a handle with a loop of nichrome or platinum wire attached to transfer specimens or microorganisms from one medium to another and to streak (spread) the material that was transferred.

medium—substance used to provide nutrients for the growth and multiplication of microorganisms; plural is *media.*

microbiology—study of living organisms that can be seen only under a microscope.

mycelium—one of the threadlike filaments that constitute the vegetative structure of a fungus; plural is *mycelia.*

mycology—study of fungi, yeast, and molds.

normal flora—organisms normally present and for some reason able to resist the defensive mechanisms of the person but at the same time unable to invade body tissues and cause disease unless the person's resistance is lowered.

optimum-growth temperature—temperature of incubation that gives the most rapid growth during a short period of time (12 to 24 hours).

pathogenic—capable of producing disease.

petri dish (plate)—shallow, round dish made of glass or plastic with a cover; used to hold solid media for culturing bacteria.

susceptibility testing—placement of discs containing antibiotics onto culture of microorganisms to determine which antibiotics would prevent or retard growth of that microorganism.

symbiosis—living together of two or more organisms in a more or less mutually beneficial state.

Microbiology is the study of living organisms that can only be seen under a microscope. These organisms include bacteria, fungi, yeast and molds, protozoa, and the viruses. A few large medical centers separate the microbiology department into bacteriology, mycology, parasitology, and virology.

Normal Flora vs. Pathogens

Microorganisms are found in large numbers on and in the human body. Under normal conditions, these microorganisms are harmless. They are called part of the ***normal flora.*** Organisms that are part of the normal flora are those that are normally present and for some reason able to resist the defensive mechanisms of the person. At the same time, they are unable to invade the body tissues, unless the person's resistance is lowered. Therefore, the person and the microorganisms exist in *symbiosis*, or living together in a mutually beneficial state. Normal flora of one part of the body differ greatly from the normal flora of another part. But as long as the person's resistance is maintained at a sufficiently high level, the bacteria that make up the normal flora do no harm. However, from time to time, organisms invade tissues and become pathogenic. It is the responsibility of the microbiology department to grow, isolate, and identify the microorganism that is causing the disease condition.

Collection of Specimens

Materials most commonly cultured are urine; spinal fluid; tissue fluids; stools; specimens collected from the nose, throat, wounds, abscesses, or genitalia; and materials suspected of containing fungi or acid-fast organisms.

Generally, there is a joint effort by the nursing staff and the laboratory personnel to obtain the proper specimen for analysis. In hospitals, nursing usually has the responsibility of collecting the specimen, labeling it properly, and sending it to the lab. In the clinic or physician's office, the medical laboratory personnel may often be part of the collection team. But no matter who the collection agent is, the specimens submitted for examination must be collected by aseptic technique into appropriate sterile containers. The specimens must then be carried immediately to the laboratory.

It is important that specimens not be left sitting around the nursing station or the laboratory because the pathogenic microorganisms may become overgrown by contaminants. It is the responsibility of the laboratory personnel to see that the specimen is inoculated as quickly as possible onto the correct media. Generally, if a specimen cannot be processed within an hour or less after it is collected, it should be refrigerated to prevent contaminant overgrowth. However, if the specimen is collected in transport media, it can wait.

Quality control in microbiology begins with specimen collection. It then continues through logging, processing, cultivating, isolation and identification, susceptibility testing, and reporting. Below are some general guidelines to be observed in the collection of specimens:

1. Rectal swabs should be placed in GN broth.
2. Swabs for *Neisseria gonorrhoeae* should be inoculated on Thayer-Martin or Transgrow at the time of collection.
3. Aspirates and washing should be collected into sterile containers and promptly delivered to the lab. They should be processed immediately to ensure recovery of the pathogens and prevent overgrowth of contaminants.
4. Spinal fluid (CSF) must be collected into a sterile screw cap tube and sent immediately to the lab. This is already an excellent medium for bacterial growth, and it should not be refrigerated.
5. Blood specimen should be collected from a venipuncture site that has been properly disinfected with alcohol and iodine. The blood should be collected

into one bottle of SPS medium that has an anaerobic atmosphere. Some blood should also be collected into one bottle of SPS medium that has an aerobic atmosphere.

6. Urine should be either a catheterized or a midstream, clean-catch specimen collected after proper cleansing of the genital area. A sterile, wide-mouth container should be used. Specimens should be processed within 2 hours.
7. Swabs from wounds and abscesses suspected of having anaerobes should be placed in an anaerobic transport unit (prereduced swabs or evacuated, anaerobic test tubes).
8. A swab of a body part for aerobes and yeast should be placed in a sterile transport medium and delivered to the lab.
9. Biopsy specimens should be collected aseptically in a sterile container with sterile saline or phosphate buffer added to prevent drying.
10. Skin, hair, or nail clippings should be collected in sterile petri dishes or tubes.
11. Stool specimens should be collected in clean cartons and carried immediately to the lab for culture and/or ova and parasite examination. If a delay is necessary, a preservative such as 0.033 M phosphate buffer and glycerol should be added to ensure survival of *Shigella* and *Salmonella* bacteria. The use of 10% formalin and PVA will preserve trophozoites and cysts of protozoa as well as eggs, larvae, and small adult worms.

Types of Media

Because of convenience, availability, and economics, most laboratories no longer routinely prepare their own media. Media are purchased prepared, wrapped, labeled, and packaged ready to be stored in a variety of positions. The quality of the commercially prepared media is always the same when stored properly.

All biological systems, from bacteria and viruses to humans, have certain nutritional requirements. Bacteria require such things as (1) energy source, (2) carbon, (3) nitrogen, (4) several metallic elements, (5) certain vitamins and vitaminlike compounds, and (6) water.

To positively identify bacteria, several different types of media may be required. Some are listed and briefly described below.

Liquid media for growing microorganisms are called *broths.* Agar-agar is derived from seaweed. It solidifies just above body temperature but can be easily melted when heated close to the boiling point. When agar is added to a nutrient broth, the product is called *nutrient agar.* The amount of agar added determines whether the product will be a solid or a semisolid. Both plates and slants of agar are used. The agar serves as a base for growth while the nutrients are provided by the additives.

Gelatin, a protein melting at a low temperature, can also be added to nutrient broth to solidify it. This product is termed *nutrient gelatin.* Some bacteria liquefy gelatin. This characteristic can be used in identification, but makes gelatin less desirable than agar to culture bacteria.

Dyes, antibiotics, or chemicals are added to media. These media are called *selective media* because they inhibit the growth of some organisms while encouraging or selecting others to grow.

Differential media also contain dyes or indicators which give an organism a characteristic growth or reaction. The final identification of an organism may be based on its reaction to differential media.

Enrichment media are used to enhance growth of a specific bacteria while inhibiting the growth of normal flora or other bacteria which could obscure it or even inhibit its growth.

It is most important that specimens for culture are inoculated onto the correct media. Most laboratories have a chart posted that lists the various sources of specimens and the different media to be inoculated. A limited list of media most often found in laboratories includes the following: Blood Agar, Nutrient Agar, Eosin Methylene Blue Agar (EMB), Thioglycollate Broth, Biggy Agar Slants, Chocolate Agar, Thayer-Martin Agar, McConkey Agar, Salmonella-Shigella Agar (S.S.) Azide, Loefler's, Bordet-Gengou, Sabouraud, and Petragnani.

Test procedures are available to identify bacteria without resorting to differential media. Identification is based on an antigen-antibody reaction for a specific bacterium. The presence of the bacteria in a specimen will produce either agglutination or a color change depending on whether the test uses latex particle agglutination or is based on the principles of enzyme immunoassay (EIA).

Processing Specimens

At this point, the media have been prepared. The bacteriologist can now begin to process the specimens. The specimen must be labeled properly with the patient's name, room number, identification number, physician's name, date and time of collection, and exact source of the specimen. "Wound," "abscess," etc., are not complete sources. If the wound is on the "left side of the kneecap," that is what should be reported as "source."

After specimen identification is certain, the specimen information should be written into the log book or entered into the computer. A lab number for identification purposes is given to each specimen. Select the media that are appropriate for the type of specimen, streak the petri dishes of media, and prepare smears for staining. Then place the inoculated plates into the incubator upside down to prevent the accumulation of moisture on the surface of the agar.

Use of Swabs to Streak Plates

The streak plate technique is for specimens that reach the laboratory on swabs and for urines sent for culture. It is important to use the entire plate of medium. The entire procedure should be performed aseptically.

Heat the inoculating loop/needle before and after using, between streaks of a different direction, and, of course, between specimens. This prevents contamination. The inoculating loop or needle should also be heated before introducing it into any container and after the specimen has been obtained. In the past, many labs used a Bunsen burner for flaming the loop. Now most laboratories use an electric burner device to prevent problems with an open flame.

Remove the container top, and quickly pass the mouth of the container over the heating device to destroy any contaminants on the outside of the container. Remove the swab, and inoculate the medium by rolling the swab along one edge of the petri dish (see fig. 1A). Place the swab back into the container again, and replace the lid. Heat an inoculating needle. Touch to the side of the medium to cool, and streak across the area where you inoculated the medium (fig. 1B). Heat the needle again, and streak across the tip (fig. 1C) that you just finished streaking. Heat and repeat (fig. 1D). Close the petri dish, heat the needle, and return it to the usual storage place. Be certain the petri plates are labeled. Place in an incubator at proper temperature and carbon dioxide atmosphere.

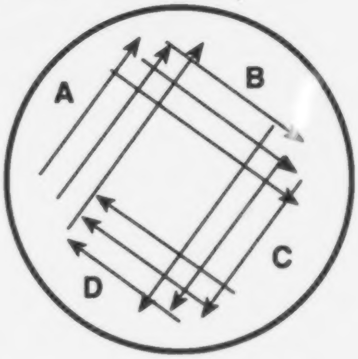


Fig. 1. The streak plate technique using a swab

Use of a Loop to Transfer a Specimen

If the specimen is urine or other liquid, use an inoculating loop to streak plates. Most loops are calibrated to contain a certain amount. Other loops may not be calibrated. Follow the procedure for your health care facility.

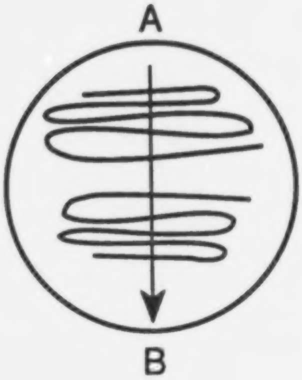


Fig. 2. The streak plate technique using an inoculating loop

Heat the mouth of the container to destroy any contaminants on the outside. Heat the loop to redness, touch the loop to the top of the liquid to cool, and then on the liquid to be transferred. Transfer the loopful to the medium, and streak the loop down the center of the petri dish (see fig. 2). Heat the loop, touch it to the medium to cool, and then streak across the entire top half of the plate. Turn the plate around, and repeat until the entire plate is streaked.

An inoculating loop can be used to transfer a specimen to an agar slant. A *slant* is a tube containing agar which was tilted before hardening to form a slanted surface. Follow the same technique for sterilizing the loop and outside of all containers. To inoculate, drag the loop across the surface of the slant with a side-to-side motion, as shown in figure 3.

Be sure that the culture is labeled. Place on the incubator shelves in oxygen, or under reduced or no oxygen to obtain optimum growth.

Factors Affecting Growth

Bacteria fall into four categories according to their oxygen requirements. *Aerobic* bacteria grow in the presence of free oxygen. *Anaerobic* bacteria grow only if there is no oxygen. *Facultative anaerobes* grow in either the absence or presence of free oxygen. *Microaerophilic* bacteria grow if only very small amounts of free oxygen are present. Refer to figure 4.

The cultivation of anaerobes requires special attention and equipment. The equipment may be as simple as a candle jar. This is a jar with a tight-fitting lid, large enough to hold a petri dish and a candle. The culture is placed in the jar, the candle is lighted, and the jar lid is fastened securely. As the candle burns out, an atmosphere containing 3 to 5% carbon dioxide results.

Elaborate, sophisticated, and expensive CO_2 incubators that strictly control the amount of oxygen and carbon dioxide present are used in

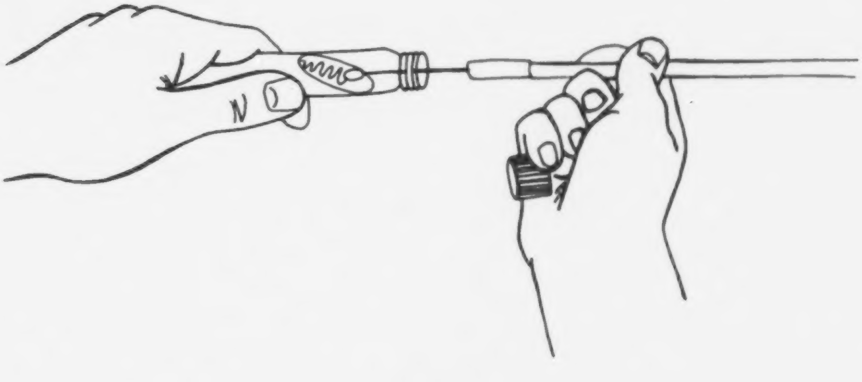


Fig. 3. Streak technique for a slant tube

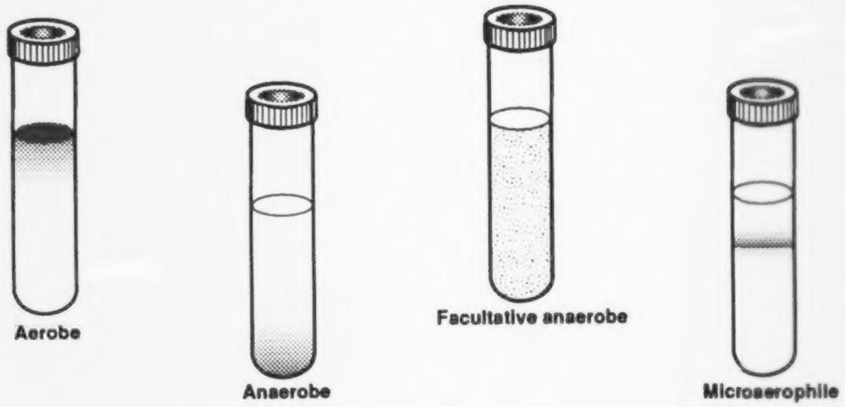


Fig. 4. Oxygen requirements of bacteria

larger laboratories. The CO_2 incubator allows air and CO_2 to enter. Controls allow the mixing of the air flow and pure CO_2 to achieve the ideal 5 to 10% content.

If only reduced oxygen content is desired, BBL (Baltimore Biological Laboratories) has a commercial gas-pack available. Simply add the required amount of water to the pack, place the petri dish and the gas-pack into a jar, close the jar, place in the incubator, and CO_2 will be released.

For those bacteria that are strict anaerobes, a gas-pack is available that has an indicator which is blue when oxygen is present and is white when the anaerobic condition has been attained. Again, the petri dishes and the special gas-packs are placed into an anaerobic jar, and then into the incubator for optimum growth at the desired temperature.

Most pathogenic microorganisms grow well at body temperature or 35°C. The optimum-growth temperature is the temperature of incubation that gives the most rapid growth during a short period of time, usually 12 to 24 hours. If the temperature rises too high, the organisms die. If the temperature drops too low, all growth ceases.

The culture is allowed to grow for 12 to 24 hours before checking. If growth has occurred, identification may or may not be possible by sight or smell.

Shapes of Bacteria and Fungi

Bacteria have many varied shapes (fig. 5). Each species has a characteristic shape. This aids in its identification. However, all can be grouped into three basic categories:

1. Coccus (pl. *cocci*) is round.
    a. Cocci in grapelike clusters are termed staphylococci.
    b. Cocci in chains are termed streptococci.
    c. Cocci in pairs are termed diplococci.
    d. Small, single cocci are called micrococci.
2. Bacillus (pl. *bacilli*) is rod-shaped or fusiform, and may have spores located at one end or in the middle.
3. Curved or spiral forms vary in shape.
    a. Spirochetes are flexible and corkscrew-shaped bacteria.
    b. Vibrios are comma-shaped bacteria.
    c. Spirilla are rigid spirals.

Susceptibility Testing

Susceptibility or *sensitivity testing* is a procedure to test several kinds of antibiotics against a culture of bacteria. If a susceptibility report is requested (see fig. 6), further growth is necessary. Isolated colonies are transferred to a large

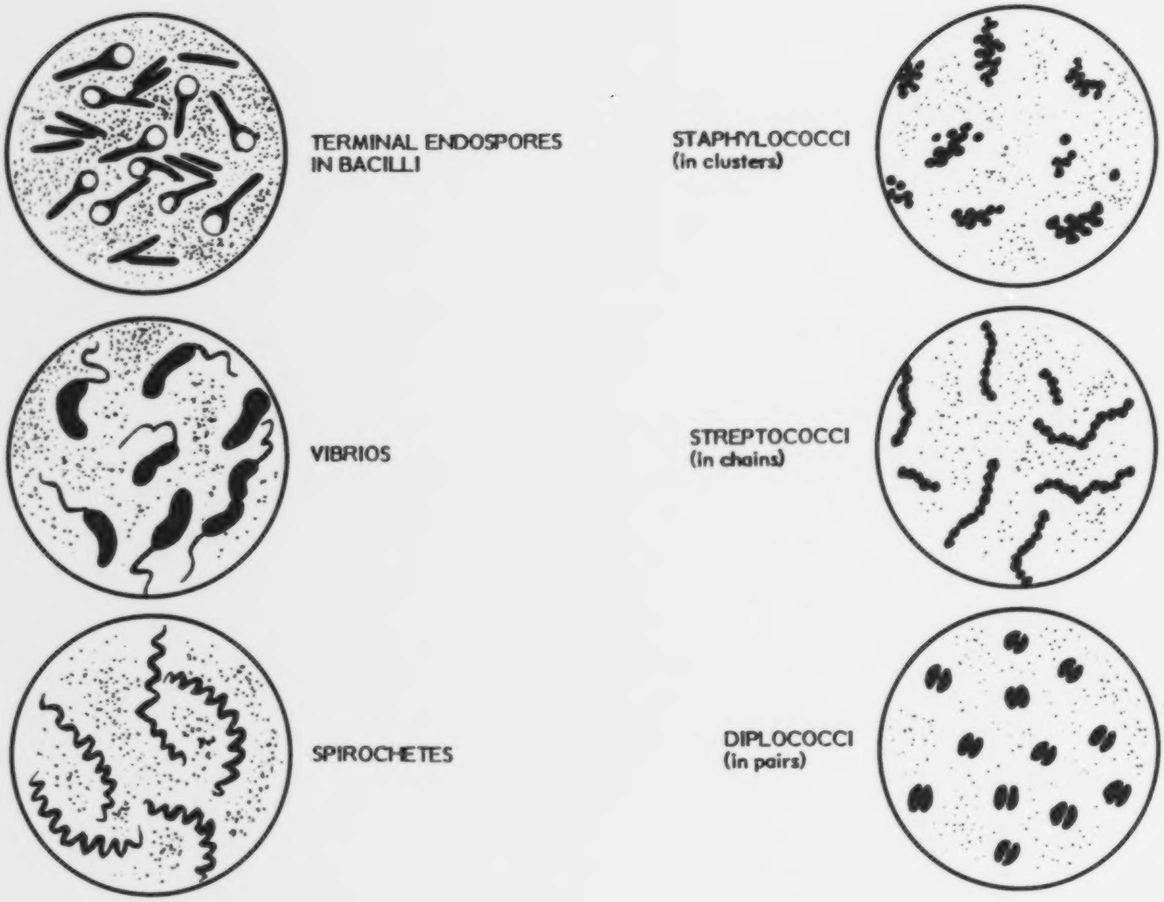
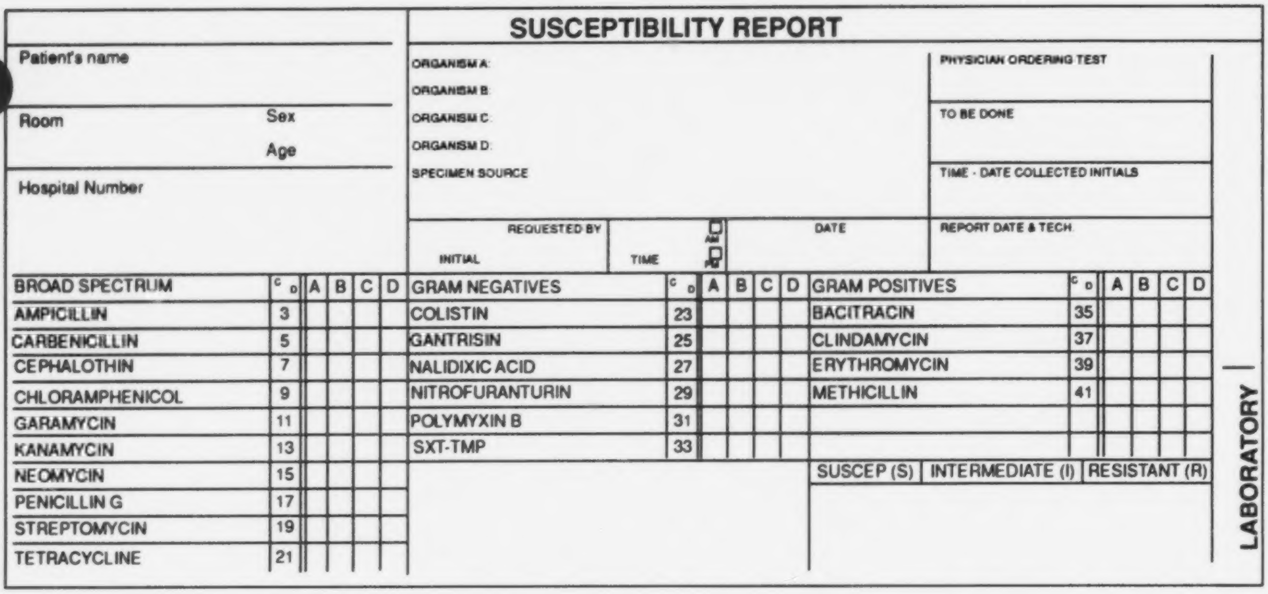

TERMINAL ENDOSPORES IN BACILLI
STAPHYLOCOCCI (in clusters)
VIBRIOS
STREPTOCOCCI (in chains)
SPIROCHETES
DIPLOCOCCI (in pairs)

Fig. 5. The appearance of bacteria



SUSCEPTIBILITY REPORT

Patient's name
Room
Sex
Age
Hospital Number

ORGANISM A:
ORGANISM B:
ORGANISM C:
ORGANISM D:
SPECIMEN SOURCE

PHYSICIAN ORDERING TEST
TO BE DONE
TIME - DATE COLLECTED INITIALS

REQUESTED BY
INITIAL
TIME ☐ AM ☐ PM
DATE
REPORT DATE & TECH

BROAD SPECTRUM	C D	A	B	C	D
AMPICILLIN	3				
CARBENICILLIN	5				
CEPHALOTHIN	7				
CHLORAMPHENICOL	9				
GARAMYCIN	11				
KANAMYCIN	13				
NEOMYCIN	15				
PENICILLIN G	17				
STREPTOMYCIN	19				
TETRACYCLINE	21				

GRAM NEGATIVES	C D	A	B	C	D
COLISTIN	23				
GANTRISIN	25				
NALIDIXIC ACID	27				
NITROFURANTURIN	29				
POLYMYXIN B	31				
SXT-TMP	33				

GRAM POSITIVES	C D	A	B	C	D
BACITRACIN	35				
CLINDAMYCIN	37				
ERYTHROMYCIN	39				
METHICILLIN	41				

SUSCEP (S)	INTERMEDIATE (I)	RESISTANT (R)

LABORATORY

Fig. 6. Susceptibility report form

petri dish of nutrient agar. The entire plate is streaked heavily. Antibiotic discs are then placed onto the surface of the streaked agar plate. Many laboratories have commercial dispensers for the antibiotic discs. The discs must be so firmly placed that they will not fall off when the petri dish is incubated upside down. All cultures on petri dishes are incubated upside down so that the moisture that forms inside the plate will not fall onto the surface of the culture.

The following procedure is standard for susceptibility testing:

1. Select four or five colonies of the organism to be tested.
2. Transfer these colonies by touching the top of each colony successively with the same loop to a tube containing 3 to 5 ml of Mueller-Hinton or trypticase soy broth.
3. Incubate the tube at 35°C long enough (2 to 8 hours) to produce an organism suspension with moderate cloudiness.
4. Dilute the broth culture with sterile saline or broth to obtain a turbidity that matches a prepared standard.
5. Dip a sterile cotton swab into the adjusted inoculum.
6. Lift the swab above the fluid level, and rotate the swab against the inside of the tube to remove as much excess inoculum as possible.
7. Streak the entire agar surface with the swab evenly in three directions.
8. Allow the inoculated plate to dry for 3 to 5 minutes with the plate closed.
9. Place discs on the agar with a dispenser or sterile forceps. With sterile forceps, press discs down gently on the agar to ensure even contact.
10. Incubate plates immediately.
11. Read plates after 16 to 24 hours of incubation.

Report as sensitive or resistant. *Sensitive* means that the bacteria were killed or had growth inhibited by the antibiotic. This is indicated by a clear zone around the disc. *Resistant* indicates the antibiotic had no effect on the bacteria, which grew up to the edges of the disc.

Large laboratories use automated methods for susceptibility testing. Various antibiotics in several concentrations are inoculated and incubated with the bacteria under study. A special

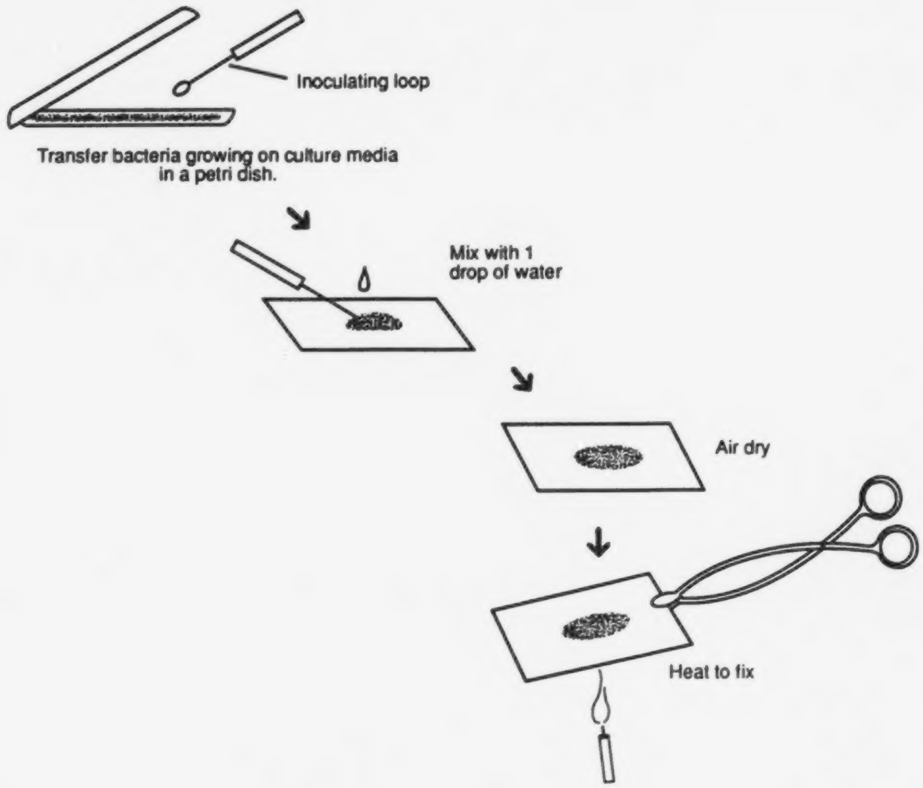


Fig. 7. Preparing a smear

instrument scans for growth, and a report is printed out.

Preparation of a Smear

One way to identify bacteria is to make a smear on a slide, stain the slide, and study the organism under the microscope. A smear can be made directly from a swab or from a culture with the aid of a loop. Frequently, two swabs are taken, with one used for a smear and one for a culture. The swab for the smear is rolled along the slide leaving a film. The slide is air-dried and heat-fixed before staining.

To prepare a smear from a culture, use the technique outlined here and shown in figure 7.

1. Place a drop of sterile water on a slide.
2. Heat a wire inoculating needle to redness. Cool.
3. Lift the petri dish lid with one hand, and remove a sample of the organisms with the loop held in the other hand. Close lid.
4. Add the sample to the water on the slide and mix, spreading thin, to form a one-inch circle. Be sure to heat the loop before setting it aside.
5. Air-dry the slide.
6. Fix organisms to slide by heating rapidly about three times. Use tongs to hold slide. Avoid overheating and cracking slide.

Gram Stain

A very important part of the bacteriological study is the Gram stain. Bacteria are differentiated according to whether they are Gram-positive (stain blue) or Gram-negative (stain red). Examination of smears stained by this technique reveals both the morphology and the Gram reaction of the bacteria. In the staining technique, the bacteria are treated with the following solutions: crystal violet, iodine solution, alcohol (decolorizing solution), and safranin or some other red counterstain. All bacteria take up the crystal violet. The iodine fixes the stain to the Gram-positive bacteria. This keeps the stain from washing off when the decolorizing solution is added. When the counterstain is added, the Gram-positive organisms do not stain red since they are already stained. The Gram-negative bacteria lose the blue color during the decolorizing process, so they stain red. The procedure below, or some modification due to manufacturer's instructions, should give a satisfactory Gram stain:

1. Prepare the slide with the smear, and allow to air-dry. Then fix the smear to the slide by heat. Do not overheat.
2. Cover smear with crystal violet for one minute.
3. Wash with tap water for 5 seconds. Drain.
4. Pour iodine solution onto smear, and leave for 1 minute.
5. Wash with tap water for 5 seconds. Drain or dry by blotting.
6. Decolorize 30 seconds with alcohol.
7. Blot dry.
8. Counterstain with safranin for 10 seconds.
9. Wash with tap water, and blot dry.

Times may vary slightly, but the order is always the same. Observe slides using oil-immersion objective of microscope after locating stained area using low power. Note appearance of Gram-negative and Gram-positive organisms.

Acid-Fast Stain

Another staining procedure that is widely used is the acid-fast stain, which classifies bacteria into acid-fast and non-acid-fast. Acid-fast organisms contain a large amount of fatty and waxy substances in the cell. Once the dye penetrates the cell (assisted by heating), it is not easily removed by the decolorizing agent. All acid-fast bacteria are also Gram-positive. Follow this procedure for performing an acid-fast stain:

1. Cover fixed smear with carbol fuchsin, and gently heat the slide (slow steaming should occur) for 3 to 5 minutes.
2. Wash with tap water.
3. Decolorize with acid-alcohol until only a small amount of red dye remains.
4. Wash with tap water.
5. Counterstain with methylene blue for 30 seconds.

The acid-fast bacteria will appear red. The non-acid-fast bacteria will appear blue.

Fungal Infections

The patient with a skin infection caused by a fungus requires special attention. It is not always satisfactory to send a sterile swab to the laboratory after simply swabbing the skin area. The infected skin should be scraped with a sharp object such as a knife blade or glass slide. Care is taken to avoid injury to the patient.

The scrapings should be placed in a small amount of potassium hydroxide (KOH). This dissolves the debris and softens the epithelial cells so any fungus structures that are present can be easily observed. A thorough search is made microscopically for hyphae, yeast, and spore forms. *Hyphae* are filamentous or thread-like pieces of a mycelium. *Yeast* are unicellular fungi that do not have mycelia. *Spores* are resistant, primitive, reproductive bodies.

Whether or not fungi are observed, a fungus culture should be prepared. Fungus cultures are different in that fungi grow both at room temperature and at 35°C in the incubator. Fungus cultures should be inoculated onto media such as Sabouraud dextrose agar. If contaminants are suspected, the addition of penicillin and streptomycin will prevent the growth of the contaminants but will allow the pathogenic fungi to grow. All work with mycelia should be done under the hood to prevent breathing of spores.

Fungi are more easily identified if they are stained. The staining procedure most often used for fungi is called the Lacto-Phenol Cotton Blue stain. To stain fungi, follow this procedure:

1. Place a small drop of the Lacto-Phenol Cotton Blue stain on a slide.

2. Use a heated needle to pick up fungus from an agar plate or directly from a scraping, and place it into the drop of stain.
3. Place a cover slip over the stained fungus, and allow it to stand for 10 minutes.
4. Examine with the low-power objective of the microscope.

Typical fungus infections include ringworm, athlete's foot, barber's itch, monilia infections, and those causing systemic infections, such as blastomycosis, histoplasmosis, and coccidiomycosis.

Summary

This unit on microbiology presents methods for collecting, inoculating, streaking, incubating, and identifying different types of pathogenic microorganisms. Types of culture media are discussed. Procedures for staining are outlined.

9

Chemistry

Objectives: After completing this unit, you will be able to—

1. *discuss methods for providing reagents and water;*
2. *list the parts of a photometer;*
3. *distinguish between manual procedures and automated procedures;*
4. *describe two types of technology used in automated equipment;*
5. *list at least five general categories of test procedures;*
6. *explain how enzyme assay is used to diagnose abnormal conditions;*
7. *describe the uses of electrophoresis; and*
8. *point out the reasons that therapeutic drug monitoring is necessary.*

Terms and Definitions

absorbance—light absorbed by a substance during photometry; also called *optical density (O.D.).*

automation—development and use of instruments to complete a testing process without human intervention.

enzyme—a biochemical catalyst within the body; produces chemical changes without being changed itself.

galvanometer—device for measuring electric currents.

mean—halfway between two extremes.

percentage of transmittance—the portion of light that passes through a substance during photometry.

photoelectric cell—device to detect light and convert it into electricity.

photometer—device that measures light.

POL—physician's office laboratory.

range—the difference between the lowest and highest observations.

reproducibility—the ability to repeat a procedure time after time with exactly the same results.

shift—results that are steady but on one side of the mean.

spectrophotometer—device that measures light intensities in different parts of the spectrum.

standard solution—a solution containing a known amount of substance.

trends—values that go steadily up or steadily down several days in a row.

variability—when measurements or results disagree but are still within acceptable range.

Chemistry is the department where the contents of blood, urine, gastric juice, cerebrospinal fluid, and other body fluids are analyzed to determine whether they are normal or how far they vary from the normal.

Using Reagents

Most clinical laboratory reagents are purchased prepared and ready to use. However, if you are preparing reagents, you must remember certain precautions. For example, when working with acids, always add acid to water in small amounts with mixing until the proper concentration is reached. **Never add water to acid.** Some acids, such as concentrated sulfuric acid, release large quantities of heat when diluted. Adding water to this acid will release enough heat to cause the water to boil and splatter and perhaps splash the acid on the person making the dilution.

Most reagents are supplied as dry powder or crystalline powder. Each of these must be diluted in water. Generally, solutions are prepared by adding the required quantity of powdered reagent to a small amount of water. They are then carefully mixed to dissolve. Finally, enough water is added to bring the mixture to the final required volume and mixed. Always follow the manufacturer's directions on the original label for making solutions. Refer to figure 1 for directions on diluting reagents.

All prepared reagents including acids should be stored in the original container. Every bottle must have a legible label. It is best to keep the original label, but replacement labels must be an accurate, identical copy of the original.

Water

All laboratories need a source of pure water. Usually, water is supplied by companies who sell bottled water. Two methods of purification are presently used, distillation and deionization.

In the *distillation* process, tap water is heated to boiling, forming steam. Steam is condensed to water and collected in another vessel. Impurities are left behind in the boiling vessel.

For *deionization*, tap water is passed through a cylinder or a series of cylinders containing tiny beads. The beads are mixtures of resins containing both negatively and positively charged ions which can be exchanged with specific ions in the water as it flows through. This resin exchange capacity is limited. A dye may be present in the resin column which indicates when its exchange capacity is exhausted. A special cylinder may be added to trap particulate matter and bacteria.

Sometimes purification methods are combined. Distilled water is passed through a deionization column to remove any materials that condensed with the water. Deionized water is distilled to remove any un-ionized impurities and to destroy pathogens with heat.

Containers must have a label since saline and other solutions cannot be differentiated otherwise. All water containers must be capped to prevent dust, contaminants, and atmospheric gases from entering the water. The use of a plastic container prevents impurities from glass from dissolving in the water.

Manual and Automated Procedures

In the medical laboratory there are many different acceptable procedures for tests. The procedures given in this publication may be unlike those in the laboratory where you work or observe. For example, blood analyses may be done manually or with the multisample analyzers (SMA). Students must consult with their supervisor to find out which laboratory procedures they may actually perform, which they may set up but must have supervision to evaluate completely, and which they may only observe.

Automation is now used to some extent in almost all laboratories. ***Automation*** is the development and use of instruments to complete a testing process without human intervention. The automated system must include some kind of sampling device, a means to add the needed reagents and sample, incubating modules, modules to measure, and a recording device. The measuring module is usually a colorimeter. These same steps are followed in manual procedures. In fact, almost any manual method can be adapted to an automated system.

The human element is still vital in laboratory testing even though automation may seem to diminish the importance of the laboratory person. The technologist must still prepare serum, load sample cups, and, most important of all, be constantly aware of the validity of the automated system.

Factors that determine the extent to which a given laboratory uses automated procedures are the size of the laboratory, economic feasibility, the number of tests performed, and the training and preference of the medical technologist.

Spectrophotometers

Devices that measure light are called photometers. Figure 2 shows two basic types. Photometers or spectrophotometers are used in

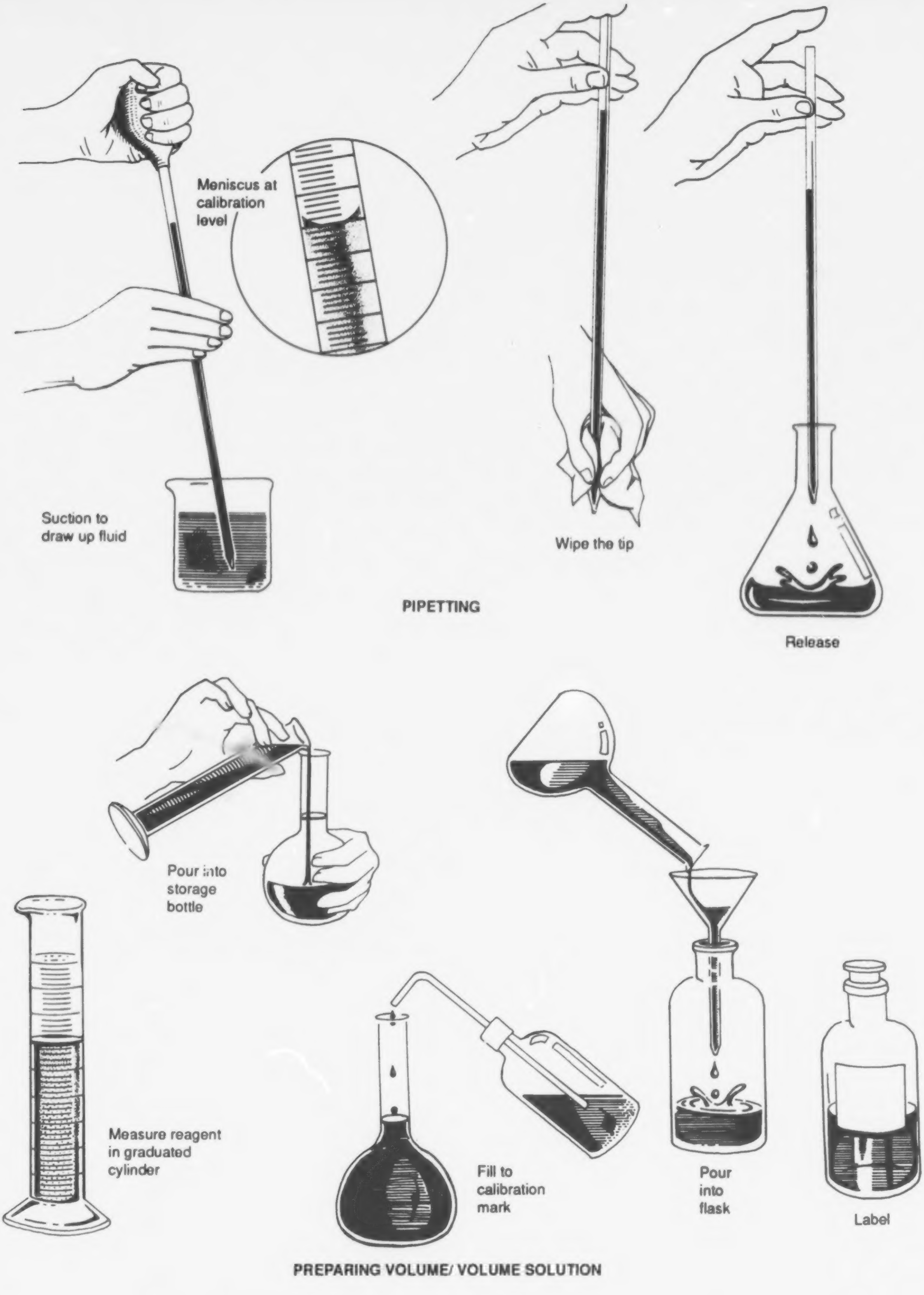
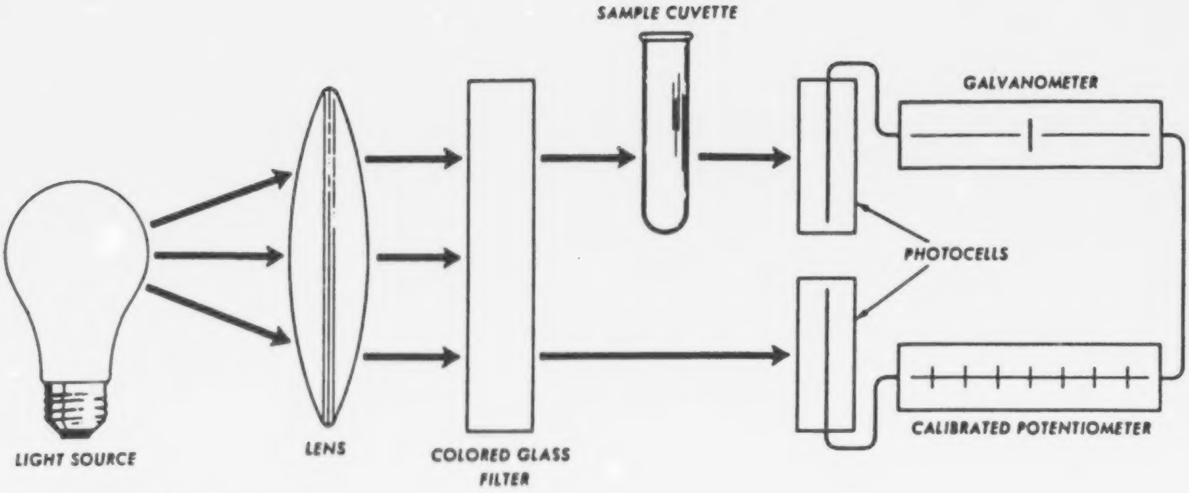
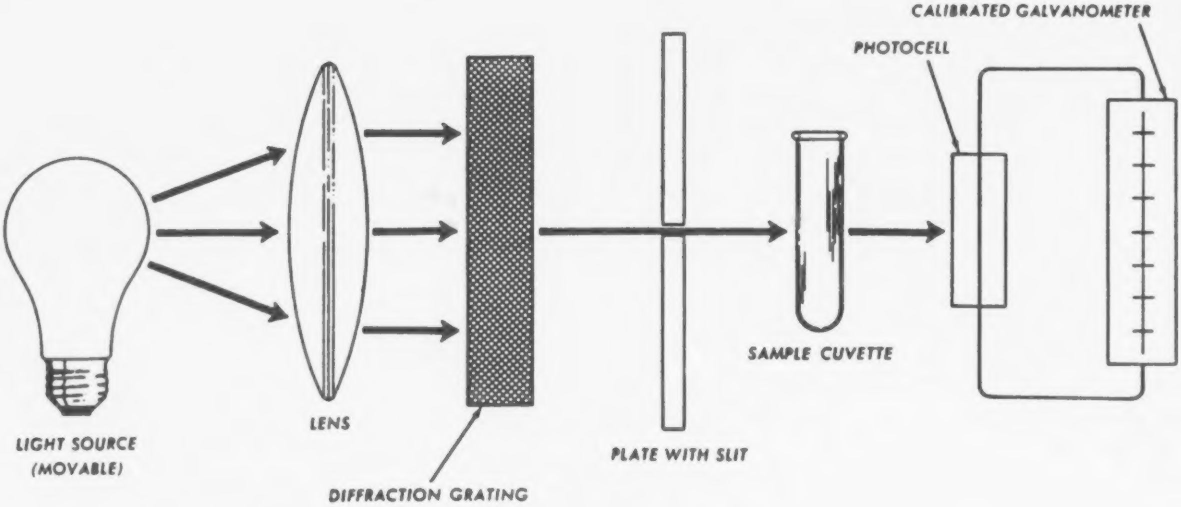

Meniscus at calibration level
Suction to draw up fluid
Wipe the tip
Release
PIPETTING
Pour into storage bottle
Measure reagent in graduated cylinder
Fill to calibration mark
Pour into flask
Label
PREPARING VOLUME/ VOLUME SOLUTION

Fig. 1. Preparing solutions using a pipette, graduated cylinder, and volumetric flask


SAMPLE CUVETTE
GALVANOMETER
PHOTOCELLS
CALIBRATED POTENTIOMETER
LIGHT SOURCE
LENS
COLORED GLASS FILTER

Double-cell filter photometer


CALIBRATED GALVANOMETER
PHOTOCELL
SAMPLE CUVETTE
LIGHT SOURCE (MOVABLE)
LENS
PLATE WITH SLIT
DIFFRACTION GRATING

Single-cell spectrophotometer

Fig. 2. Types of photometers

determining the presence and quantity of chemical substance in the blood. The substances in the blood can be treated in such a manner as to yield a color that can be measured quantitatively. Visible light is a result of energy changes of electrons in the outer shell of atoms. Infrared radiation is caused by vibrations of the molecules. Ultraviolet radiation is due to energy changes of electrons in the inner shells of the atoms.

Photometers are classified according to the type of wavelength selector used. Wavelength selectors may be colored glass filters or diffraction grating (a thin piece of plastic ruled at regular intervals). Photometers must have a light source, a focusing lens, a wavelength selector, a photocell to convert the light to electricity, and a device to measure the electricity produced. The voltage supply to the light source must be constant.

To use photometry, a reaction must take place between the substance to be measured and a reagent, producing a color change. The determination of the concentration of the substance is based on Beer's law. Beer's law states that the color intensity is directly proportional to the density. When a reagent or chemical is added to a substance to produce a color reaction, the intensity of the color is proportional to the amount or concentration of the substance present. The portion of the light that passes through a substance in the photometer is the *percentage of transmittance.* The rest of the beam of light is absorbed by the substance. This amount is measured as *absorbance* or *optical density.* Transmittance decreases as the concentration of the substance rises. Absorbance tends to increase with concentration. A photometer can read either of these. In either case, the unknown substance must be compared with a standard solution. A *standard solution* is a solution containing a known amount of substance.

The filters in the photometer are used to remove interfering colors and leave one color of a certain wavelength. A diffraction grating can provide the same function. The grating has a highly polished surface with numerous lines on it to break up white light into the colors of the spectrum. The slit is adjusted to select a specific wavelength.

The most important thing to remember is to be certain the correct wavelength and selector are used for each test. The machine is set at Zero Absorbance or 100% Transmittance with the blank, and matched cuvettes (sample holders) are used. From readings of the spectrophotometer, calculations are made to determine the value of the sample that is tested.

Spectrophotometers can be a separate instrument used for manual procedures in offices or small clinics. They can be incorporated into an automated system in a large laboratory. All photometers should be protected with a dust cover when not in use. Cuvettes for manual procedures should be absolutely clean and free from scratches. Their outside is wiped clean, after a sample is poured in, and they are held by the upper edge only for insertion into the photometer.

Types of Technology

The number of laboratories in doctors' offices is increasing. These are called *POLs* or physician's office laboratories. Sometimes, one technologist will be employed by several physicians and travel from laboratory to laboratory during one day or from day to day during a week. On-site testing is becoming popular within large hospitals to speed treatment of patients in emergency situations. Satellite laboratories can be established for intensive care units and the operating room away from the central lab. Laboratory procedures carried out in these areas are termed *point-of-care testing.*

The changes to POLs and point-of-care testing have been made possible by the development of microcomputers and microelectronics for use in automated testing equipment.

At present, two types of technologies are used in automated clinical laboratory equipment—continuous flow analysis and discrete analysis. During *continuous flow analysis,* both samples and reagents constantly flow through the equipment. They are added at specified time intervals. Samples are separated by either reagents or air bubbles.

Patient samples are placed in individual containers for *discrete analysis*. The individual containers eliminate mixing of samples. Two procedures can be employed for this type of analysis: centrifugal analysis and solid-phase analysis.

For discrete *centrifugal* analysis, the patient sample and reagents are brought together in a container. Centrifugal force is used to mix them during the reaction time period. The test results are obtained by a reading from a spectrophotometer within the analyzer.

When *solid-phase* analysis is used, the patient sample is added to dry reagents on a slide or strip. The reagents occur in layers which react with the sample for testing. The area where the reagents are located is called the test patch or test area or reagent patch. The bottom support layer on the strip may be transparent so that the instrument can read the test results. A different strip is used for each type of test and is identified by the machine through a code on the strip. After a reaction period, the machine reads assay results, calculates, and displays its findings.

Quality Control

Any time a test result is reported, the technologist, physician, and patient want to be certain the report is correct. Today all laboratories are required by regulatory agencies to use some form of quality control to make sure that test results fall within acceptable limits.

Quality control must exist at each step and starts with specimen collection. Careless work by any member of the laboratory team or malfunction of instruments can produce results that are unacceptable. Equipment should be serviced and checked regularly. Water baths must have water at sufficient levels. Reagents must be checked to be sure they are fresh and uncontaminated. The temperature of water baths and refrigerators must be measured regularly. A complete and current record of these checks should be kept in an easily accessible place in the laboratory.

Control sera serve to check on temperature, pH, instrumentation, correctness of wavelength, reagents, technique, and possible deterioration of reagents. Control samples and standards must be run through regular test procedures. These can be plotted. Trends or shifts over a period of several days can be easily spotted. A *trend* shows values that go steadily up or steadily down several days in a row. A *shift* has results that are steady but on one side of the mean. By plotting controls, you can tell at a glance whether controls are within range. The *range* is the difference between the lowest and highest observations. The *mean* is halfway between two extremes. Quality control allows the laboratory to closely monitor its reproducibility, accuracy, and variability.

Quality control charts and data provide information that allows the laboratory to spot potential problems before patient results become meaningless to the physician. Quality control allows the laboratory to take immediate action before a test is completely out of control. The medical laboratory worker may be assigned the task of checking and recording temperature readings, water levels, and countless other checks that are a vital part of quality control. Figures 3 and 4 are examples of a quality control chart and a maintenance chart.

General Tests

The general tests described here are routinely performed in the clinical chemistry laboratory. Assay may be performed manually or by automation.

Glucose or blood sugar. The oxidation of glucose provides energy for various biochemical processes. Diabetes mellitus is a disease that results from the body's inability to use glucose properly. Blood glucose determinations are used for diagnosis and treatment, as well as in routine physical examinations and health screening programs. Some samples are collected fasting, and others are postprandial (after eating). Tests done on blood and urine over a timed period of several hours are called a glucose tolerance test. This test aids in diagnosing hypoglycemia as well as hyperglycemia.

Blood urea nitrogen (BUN), uric acid, creatine, and creatinine. The kidneys excrete these chemicals as waste. An increase of these chemicals indicates that the kidneys are unable to ex-

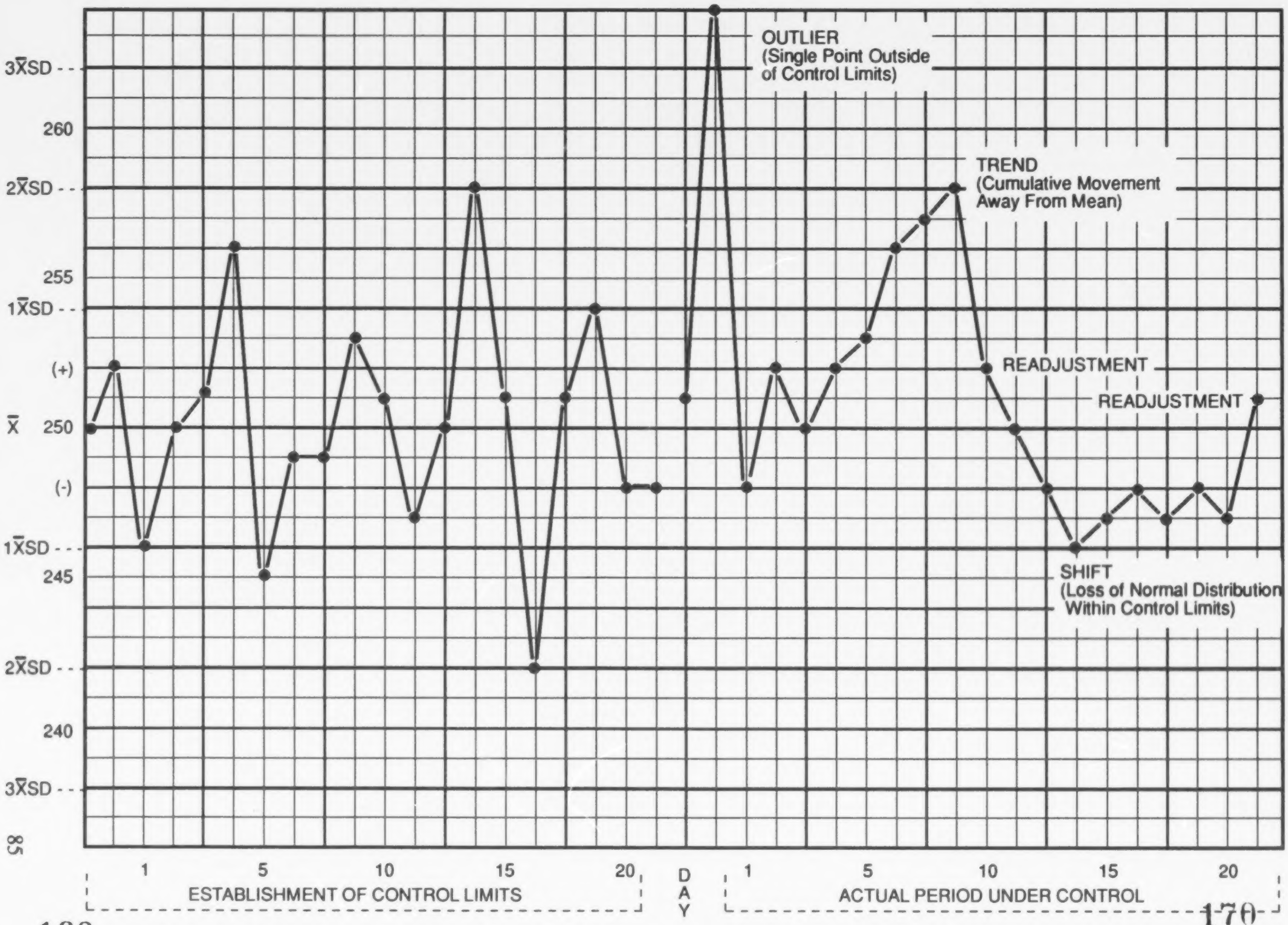


Fig. 3. Sample quality control chart

MANUAL CHEMISTRY MAINTENANCE LOG

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			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
	Date																																
	Initials																																
1	Prepare controls																																
2	Discard old specimen tubes (save day before only)																																
3	Check for refrigerated specimens																																
4	Clean counter space with 10% chlorox																																
5	Check temperatures and record	37° waterbath																															
		37±1°/ division	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –
		Refrigerator (white)																															
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		Freezer																															
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		Perkin-Elmer																															
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		Sensi-tex Incubator																															
		37±1°/ division	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –
		Walk-in Refrig.																															
		5±2°/ division	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –
6	Empty water bottle in refrigerator as required																																
7	Check spectrophotometer absorbances	360 nm																															
		36±1°/ division	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –
		572 nm																															
		11±1°/ division	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –
		620 nm																															
		98±1°/ division	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –
8	Clean micro-centrifuge with 10% chlorox																																
9	Clean 37° waterbath																																
10	Fill 37° waterbath		X	X	X				X	X	X	X	X	X	X	X	X	X	X	X				X	X	X	X	X	X	X	X	X	X
11	Clean centrifuges with 10% chlorox																																
12	Replace centrifuge brushes (as required)																																
13	Clean work area with 10% chlorox																																

Fig. 4. Manual Chemistry Maintenance Log

crete adequately. Uric acid testing is used primarily for determination of gout.

Proteins (albumin and globulin). Proteins make up the support tissue of animals, protect us from disease in the form of antibodies, and are the basis for the basic functions of life. The ratio of albumin to globulin (A/G ratio) is used in the diagnosis of liver and kidney disease.

Bilirubin and bromsulfalein (BSP). These are tests of liver function. Bilirubin is formed when hemoglobin is broken down. Only an increase is significant.

Triglycerides, cholesterol, phospholipids, and fatty acids (total plasma lipids) determinations. The body lipids (fatty acids) relate to fat metabolism by providing stored energy. They also form part of the structural units of cell membranes and hormones. Cholesterol and triglyceride tests can be used to indicate susceptibility to atherosclerosis and problems with fat metabolism. Cholesterol can be broken down for testing into high-density lipoprotein cholesterol (HDL) and low-density lipoprotein cholesterol (LDL). The HDL is considered "good" because it is broken down in the liver. The LDL is considered "bad" because it is the type stored as fat in the tissues. Physicians use the ratio of the LDL divided by the HDL to determine the heart attack risk factor. The larger the-ratio, the greater the risk.

Calcium, phosphorus, and magnesium. These are important in blood coagulation and kidney function. An increase in one results in a decrease in the other. A delicate balance must be maintained for proper functioning of body processes.

Electrolytes (chloride, sodium, potassium, and carbon dioxide). Chloride is important in the maintenance of osmotic pressure and is the anion present in the largest amount in the body fluids. Sodium is the cation present in the largest amount in extracellular fluid. Potassium is the largest intracellular cation. These, along with carbon dioxide, maintain a balance of the blood pH. The normal range of the blood pH is 7.35 to 7.45. This range is narrow, and deviations result in acidosis, alkalosis, or death.

Enzymes

Enzymes are biochemical catalysts within the body that allow the body to carry out its chemical reactions at body temperature and at a pH compatible with life. They are not changed during the reaction. Tests are most commonly done for the following enzymes:

- **Amylase** breaks down starches into sugars. Abnormal results indicate problems with the pancreas, salivary glands, or the liver.
- **Lipase** is furnished by the pancreas to break down fats. Elevations indicate pancreatic disorders.
- **Alkaline phosphatase** is important in the formation of bone and is elevated in bone disease and also jaundice caused by obstructions.
- **Acid phosphatase** is elevated in cancer of the prostate, particularly if the cancer has spread to other parts of the body.
- **Transaminases** are elevated when there is deterioration of tissue in an organ.
  - *Serum glutamic oxalacetic transaminase/aspartate aminotransaminase (SGOT/AST)* is present in heart muscle, liver tissue, and other organs. Destruction of cells in these areas causes an increased release into the blood.
  - *Serum glutamic pyruvic transaminase/alanine aminotransaminase (SGPT/ALT)* is found in liver tissue.
  - *Lactic dehydrogenase (LDH)* is used to diagnose cardiac and liver disorders, anemias, and pulmonary infarction.
  - *Creatine phosphokinase (CPK)* is used in diagnosis and prognosis following heart attacks.

Many test procedures are ordered so infrequently that it would not be economically feasible to have on hand at all times everything necessary to perform the procedure. These tests are often sent to reference laboratories: Dioxin, Dilantin, Folic Acid, and B_{12} requests. Hepatitis B Antigen, Insulin, Serum Iron and

Iron-Binding Capacity, hormone studies, Lithium, Prolactin, Renin, Rubella, VMA, and Catecholamines are other tests often referred. There are many other tests that are even special procedures for reference laboratories.

Profiles

Combinations of general tests to study a specific organ or function are called *profiles*. Automated machines take a sample of a patient's serum and run from 12 to 20 tests on a very small sample. A large number of tests indicates the patient's general condition and may be a combination of several profiles. Specific profiles may be ordered for liver function, kidney function, cardiac condition, carbohydrate usage, or lipid metabolism.

Protein and Hemoglobin Electrophoresis

Electrophoresis is the movement of charged colloidal particles through an electrical field. The particles move with different velocities due to size, shape, and the electrical charge they carry. The particles are dispersed onto a stabilizing medium by the electrical current so that they can be stained and studied. The stabilizing medium can be paper strips, cellulose acetate, or a layer of agar. The quantity present can be determined by the intensity of the color in the stained portion.

Through electrophoresis, lactic dehydrogenase (LDH) can be separated into five fractions or isoenzymes. Increased isoenzymes 1 and 2 indicate heart trouble, LDH_3 indicates pulmonary infarction, and fractions 4 and 5 are associated with liver disease.

Abnormal hemoglobins can be detected by electrophoretic procedures. The patterns formed by the hemolyzed blood cells from a test sample can be matched against standard bands of known hemoglobin. Abnormal types of hemoglobin which may be identified are ***hemoglobin S***, which results in sickle-cell anemia, ***hemoglobin C***, responsible for a type of hemolytic anemia, and ***hemoglobin F***, a type of fetal hemoglobin which persists later in life leading to thalassemia. Thalassemia is an inherited abnormality causing a type of anemia.

Therapeutic Drug Monitoring

Many times physicians need to monitor the effects of the drugs they prescribe for patients. The drug level must not drop too low, or it will not have the desired effect. Too high an amount can endanger a patient. Some drugs are toxic if not taken properly. Frequent monitoring can alert the physician before problems develop. Some of the medications that require careful monitoring are digoxin, lithium, phenobarbitol, quinidine, and certain antibiotics.

Laboratories are also called on to evaluate for drugs of abuse, suicide attempts involving overdoses, and accidental ingestion of drugs by children. The tests are not run as legal evidence. Law enforcement agencies perform legal testing. The results from the clinical laboratory can give physicians indications for treatment.

Summary

Reagents and a good water supply are necessary in chemistry. Figure 1 shows techniques for preparing reagents. Automation is the use of instruments for testing. Types of automated equipment are discussed. The use of photometers is explained. Quality control must exist at each step in testing. The following procedures are discussed: general tests, enzymes, profiles, electrophoresis, and therapeutic drug monitoring.

10

The Blood Bank

Objectives: After completing this unit, you will be able to—

1. *screen blood donors;*
2. *outline the process for drawing a unit of whole blood;*
3. *describe the processing and labeling of blood;*
4. *name the four groups in the ABO blood grouping system;*
5. *explain how the ABO blood group and Rh factor are determined;*
6. *list and define components of blood;*
7. *discuss cross-matching procedures; and*
8. *explain why a Coombs Test is performed.*

Terms and Definitions

agglutinins—antibodies in plasma which react with agglutinogens on the red blood cells.

agglutinogens—antigens found on the surface of red blood cells.

autologous blood—donation of blood by an individual before surgery for his/her intended transfusion later.

components—portions of the blood separated from a single unit for use in different transfusions.

cross matching—tests performed to determine whether a donor's blood will be compatible with the recipient's blood during a transfusion; also called *compatibility testing.*

cryoprecipitate—the precipitate formed when plasma is frozen.

donor—someone who gives blood for use in a transfusion.

iodophore—a compound of iodine with a stabilizing agent to lessen irritation.

plasma—liquid portion of blood.

recipient—the person who receives blood or its components.

red blood cells—concentrated solution of blood cells used for transfusion; separated from serum by centrifugation or undisturbed sedimentation.

transfusion—replacement of whole blood or its components.

whole blood—the entire unit of blood.

Some hospital laboratories maintain a blood bank. Others depend on local or area blood banks which serve more than one hospital. All blood banks must meet standards to ensure delivery of a quality product and the safety of donors.

Transfusions are necessary to replace blood lost through hemorrhage due to trauma or surgery, destruction of erythrocytes, or the body's inability to provide its own blood supply.

Blood Donor Screening

Anyone wishing to donate blood must be carefully screened and sign an informed consent form prior to donation. Donors are not restricted by sex or race. Their age will be from 17 to 65 years. Usually donors are limited to five or fewer donations per year. At least 8 weeks must elapse between donations.

A donor card is established for each person

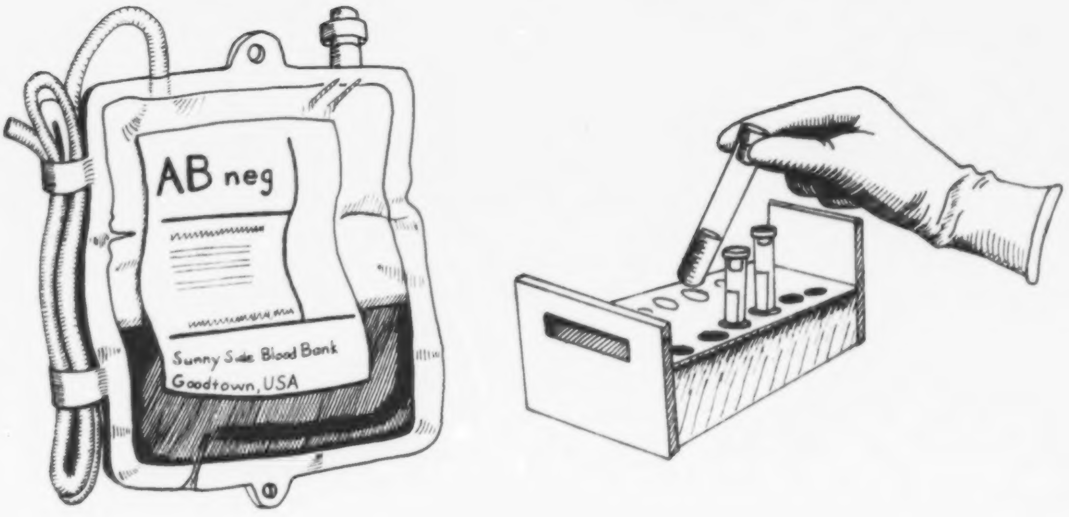


Fig. 1. Unit of blood and rack of pilot tubes

as a permanent record. The card contains personal identifying information, including a Social Security number, and the person's medical history. The date of donation and results of screening are recorded. The donor is assigned a number that is carefully entered in the blood bank's record system or data bank.

A donor can be permanently rejected for the following reasons: viral hepatitis; drug or alcohol addiction; malaria; AIDS; tuberculosis; syphilis; disease of the heart, liver, or kidney; convulsive disorders like epilepsy; cancer; blood diseases; and diabetes mellitus. A transfusion of blood or components, major surgery, and tattooing will cause deferment of a donor for 6 months. Women who are pregnant will be deferred until 6 months after delivery. A person must wait for 2 to 4 weeks after receiving a vaccination or immunization, depending on the type.

A donor is given a partial physical examination before donation. The donor must weigh at least 110 pounds. Body temperature, pulse, and blood pressure must be within normal range. The hemoglobin and hematocrit are determined and must be normal.

Donor screening must include questions that relate to AIDS, HIV (human immunodeficiency virus) infection, ARC (AIDS-related complex), and the high-risk behaviors associated with these conditions. Each donor must be given an opportunity, in confidence, to indicate that the blood drawn will not be suitable for transfusion. If significant abnormalities are detected during processing, the blood bank must notify the donor.

Collection and Numbering of Blood

Both donor and recipient must be protected when blood is collected for transfusion. The site selected for venipuncture must be free of lesions. The site is made aseptic by cleaning with soap and 70% alcohol. Finally, an iodophore is applied. An *iodophore* is a compound of iodine with a stabilizing agent to lessen irritation. A restricting band is placed above the collection site, usually a sphygmomanometer held at 40mm Hg. The needle is inserted firmly, and blood flows into a closed collecting system (plastic bag with tubing attached).

The collecting unit contains an anticoagulant. This is usually sterile, acidified citrate-dextrose (ACD) solution. The proper amount of blood must be collected to constitute a unit of blood. A unit should contain 450-500 ml of whole blood. During collection, the anticoagulant in the plastic bag must be mixed frequently with the incoming blood to prevent formation of any clots. The donor must be watched during and after collection for signs of fainting.

The needle is removed from the donor's arm with pressure and elevation applied to stop bleeding. A special tube, called a *pilot tube*, is collected after the needle is withdrawn by inserting the needle into a vacuum tube. This is used for donor testing. The collection tubing is heat-sealed into segments for use in cross-matching procedures. Refer to figure 1.

When the unit of blood is collected, it receives a unique number. All test tubes, segments of tubing, and printed records are identified by the number assigned to the unit by the blood bank. The number is in series and is logged carefully into the bank's records. Blood bank personnel must be able to locate and provide a history for any unit of blood should a question be raised later by a physician or patient.

After collection, the blood must be placed in storage. The temperature must be maintained at 1° to 6°C.

Processing Blood

Each unit of blood is tested for liver disease, different types of hepatitis, the HIV virus, and syphilis. The RPR test procedure used to detect the antibodies to *Treponema pallidum* is outlined in Unit 7. The blood group and Rh factor are determined. During processing, each unit of blood receives a label. The label must show blood group, Rh factor, type of anticoagulant present, donor identification, date of expiration, and the name and address of the blood bank.

The units of whole blood are stored in a refrigerator. Once processing is completed, the bags are arranged by ABO groups, that is, into Group A, Group B, Group AB, and Group O. These groups are divided into Rh-positive and Rh-negative. The refrigerator must be maintained at a temperature of 1° to 6°C. A recording thermometer keeps a continuous, permanent record of the temperature. Should the temperature exceed the accepted limit of 10°C, an alarm will sound during the day or at night.

Daily inspections are conducted of stored blood. Each unit is inspected for clot formation, turbidity, or color changes, such as hemolysis, and findings are recorded on the daily record of inspection. Outdated blood is discarded.

Blood units are often exchanged between banks as the need arises. Incoming blood must be added to the receiving log book or data bank. Outgoing blood is recorded also. All units must be identified as to number (donor), ABO type, Rh type, and dates of arrival and expiration.

Some people, anticipating elective surgery, may bank their own blood for future use. This blood is called *autologous blood*. Such a donation, storage, and transfusion require the consent of both the blood bank physician and the patient's physician.

The Blood Groups

Among the important factors in the blood are antigens and antibodies that determine blood types. An *antigen* is a substance that reacts with its specific antibody. Antigens are named for the type of antigen-antibody reaction they may bring about. The antigens on the surface of red blood cells are called *agglutinogens*.

Antibodies are protein substances in the plasma that react with their specific antigen. Thus, agglutinogens (antigens) will react with *agglutinins* (antibodies) to bring about agglutination. There are many different kinds of antigen-antibody reactions.

Two kinds of agglutinogens may be found on the surface of human red blood cells, called A and B. An individual may have either A or B, neither, or both. A person's blood group or type is determined by the presence or absence of these A and B antigens.

If a person has A agglutinogens on the red blood cells, than she/he will also have corresponding antibodies called Anti-B agglutinins in the plasma. Persons with B agglutinogens will have Anti-A agglutinins. Those with both A and B agglutinogens will have no agglutinins; those with neither A nor B agglutinogens will have both Anti-A and Anti-B agglutinins. Plasma could never contain antibodies against

Blood Group	Agglutinogen Red Blood Cells	Agglutinin in Serum	Cells Agglutinated
A	A	Anti-B	B, AB, O
B	B	Anti-A	A, AB, O
AB	A and B	None	A, B, AB, O
O	None	Anti-A, Anti-B	None

Fig. 2. ABO blood groups

the antigens on its own red blood cells. If so, the antigen and antibody reaction would bring about the agglutination of the person's own red blood cells. Figure 2 shows the antigens and antibodies present in the various blood types.

Blood types are hereditary and do not change during one's lifetime. The pattern of inheritance is predictable. It is possible, by determining blood types, to know whether a given person *could be* the parent of a particular child, but not that she or he *is* the parent. DNA research may provide definite proof of parentage.

The percentage of a given blood type in various ethnic groups varies. In the United States, the following percentages have been found: Type O, 47 percent; Type A, 41 percent; Type B, 10 percent; and Type AB, 4 percent.

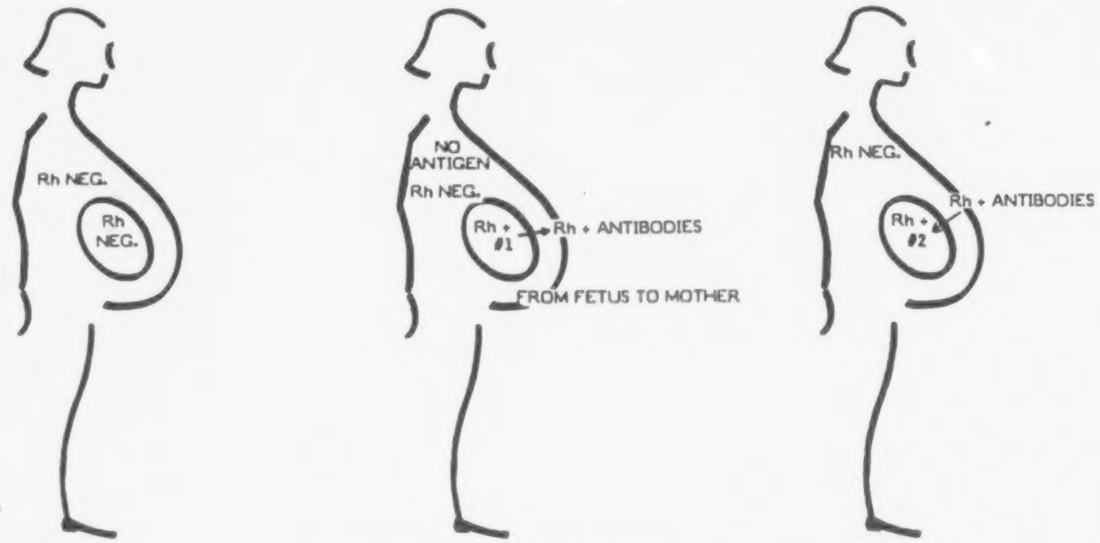
The Rh Factor

The Rh system is based on the presence or absence of a certain antigen on the erythrocytes. The factor was first discovered in Rhesus monkeys. The Rh symbol derives from their name. About 85 percent of the population in the United States have these antigens and will test Rh-positive. Those who lack the antigen are Rh-negative. Rh antigens are on the surface of the red blood cells as well as the A and B antigens. Thus, a person can be Type A, B, AB, or O and also Rh-positive or Rh-negative.

The Rh factor is of little importance except in the pregnancy of Rh-negative persons, or if they receive blood transfusions. If a person who is Rh-negative receives Rh-positive blood, that person may become sensitized to the antigen. The body is stimulated to produce specific anti-Rh antibodies. These remain in the blood. If the patient receives further transfusions of Rh-positive blood, the Rh antigen on the donor's red blood cells and the Rh antibody in the patient's blood will react, causing the red blood cells to agglutinate with serious consequences or even death.

To avoid this problem, Rh determinations are done prior to transfusion with whole blood, and Rh-negative patients are given only Rh-negative blood. Thus, there can be no antigens to stimulate the production of antibodies.

A similar reaction can occur during pregnancy, when the mother is Rh-negative and the baby is Rh-positive. The inheritance of Rh types follows genetic patterns. The child of an Rh-negative mother and an Rh-positive father could be either Rh-positive or Rh-negative. If the child is Rh-positive, it is possible for the Rh antigens to cross the placental barrier during pregnancy and cause the Rh-negative mother to produce Rh antibodies. In a subsequent pregnancy with an Rh-positive fetus, an antigen-antibody reaction between the infant's red blood cells and the anti-Rh antibodies in the mother's blood could cause the red blood cells of the infant to break down or hemolyze, releasing hemoglobin in the baby's blood. This may cause the baby to be anemic. Further damage may be caused if the disintegrating red blood cells occlude the blood vessels. This is called



NO REACTION

Rh + ANTIGEN FROM INFANT CROSSES PLACENTAL BARRIER TO BLOOD STREAM OF Rh -- MOTHER

REACTION
Rh + ANTIBODIES IN BLOOD STREAM OF MOTHER REACT WITH Rh + ANTIGEN OF FETUS.

Fig. 3. Rh factor in pregnancy

erythroblastosis fetalis or *hemolytic disease of the newborn.* It is treated by giving the baby many small transfusions of Rh-negative blood to replace the damaged blood. Refer to figure 3.

Currently, if an Rh-negative mother delivers an Rh-positive baby but has no anti-Rh antibodies, she is given anti-Rh immune globulin during the first 72 hours after delivery. These antibodies react with any fetal antigens in the mother's blood so she will not produce anti-Rh antibodies. Then, if a second pregnancy with an Rh-positive fetus occurs, there will be no Rh-positive antibodies present to cause a reaction.

Although Rh factors must be determined to provide adequate care of both mother and child, the actual numbers involved are small.

Blood Components

A unit of blood can be transfused as *whole blood.* Also, blood is separated by centrifugation into components so that one unit may benefit more than one person. The concentrated solution of cells (called *red blood cells)* goes to one person. The plasma can be frozen to form *fresh frozen plasma.* After freezing and thawing, a white precipitate is found in the container. This is called *cryoprecipitate* and contains most of the clotting factors. These blood clotting factors can be removed for use by someone with bleeding problems. *Plasma* can be given to restore blood volume or be further separated by chemical means into fractions such as gamma globulin or serum albumin. If water is removed, plasma can be dried and stored for a considerable length of time. Sterile, distilled water is used to restore plasma to a liquid state for transfusion.

Cross Matches

Blood types are important in blood transfusion because, of course, if donor blood contains antigens and/or antibodies of a different type from that of a recipient, the red blood cells will agglutinate and cause severe reaction or even death. Blood group O has no antigens. It is called the "universal donor" because theoretically the blood would not react with any cells in transfusion. In actual practice, patients are almost always transfused with their own type of blood. The Anti-A and Anti-B antibodies in the serum and other factors in the blood could cause an undesirable reaction. Figure 4 shows reactions of AB blood groups in cross matching.

When a patient needs a transfusion, the physician writes an order. The order is placed on a transfusion request form and sent to the blood bank or requested by computer networking. Blood bank personnel take a sample of the patient's blood and determine the ABO group and Rh factor. A unit of blood is selected which is the same group or from a compatible group. The selected donor unit is checked for compatibility with the recipient's blood by a process called *cross matching*. Cross matching refers to the tests performed to determine whether a donor's blood will be compatible with the recipient's blood during transfusion. A cross match involves three types of testing—incubation at room temperature with a saline suspension of blood cells, high protein (albumin) incubated with cells and serum at 37°C, and an antiglobulin or Coombs Test.

Coombs Test (Antiglobulin Test)

The Coombs is a nonspecific test used to detect weak or incomplete antibodies. It was first used to demonstrate weak Rh agglutinins. The Indirect Combs Test detects antibodies in vitro—antibodies in blood serum. Sometimes incomplete antibodies are not detected in the routine cross-matching procedures. The Indirect Coombs Test detects the presence of incomplete antibodies that might result in incompatibility. This test is also done routinely on new obstetrical patients and then periodically on Rh-negative obstetrical patients.

The Direct Coombs Test detects antibodies in vivo—antibodies attached to the red blood

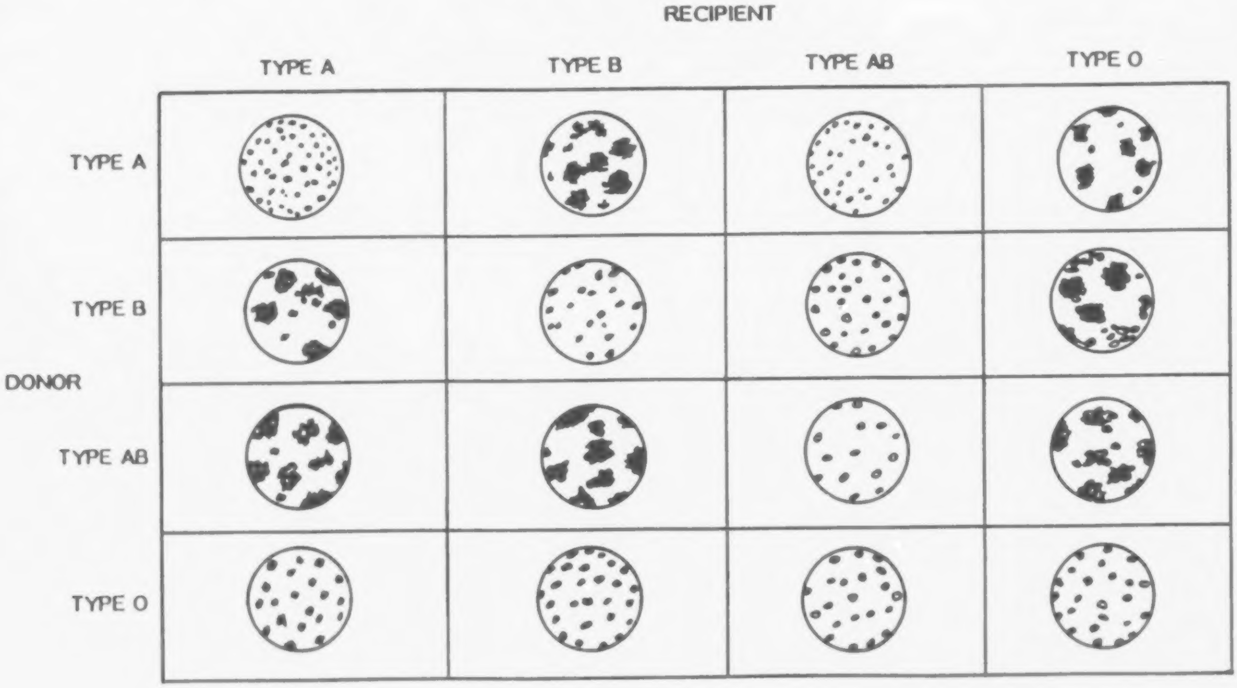


Fig. 4. Reactions of ABO blood groups in cross matching prior to transfusion

cells. In this test, the patient's red blood cells are mixed with antihuman serum. The antihuman serum is usually prepared from the blood of rabbits that have been injected with human red blood cells. Agglutination of the patient's red cells is a positive test. The Direct Coombs Test is used for the detection of hemolytic disease of the newborn, autoimmune hemolytic anemia, and in the investigation of hemolytic transfusion reactions.

Transfusions

Records are kept to show routine or special testing. After the cross match indicates compatibility, the unit of blood is released to the nursing service for administration to the patient. A careful record (transfusion release ledger) must be kept to document that the cross match was done, the time and date for the release of the unit, the personnel releasing it, and the person receiving the blood.

Summary

Donors are screened and their blood drawn in the blood bank. The units of blood are typed, carefully tested for purity, and placed in the bank for future use. Blood will be divided into its components for distribution to more than one person. When a patient needs blood, workers cross match units of blood to determine compatibility with the recipient's blood. Any reaction to a transfusion is investigated. Unusual types of antibodies are identified.

11

Histology / Cytology

Objectives: After completing this unit, you will be able to—

1. *list types and sources of specimens;*
2. *outline steps in processing tissues and cells;*
3. *mark and store slides;*
4. *use exfoliate cells to identify abnormal conditions; and*
5. *practice safety precautions unique to the pathology laboratory.*

Terms and Definitions

autopsy—examination of a dead body or tissue from a dead body to establish the cause of death.

biopsy—living tissue removed for microscopic examination to aid in diagnosis.

cytology—study of cells.

dehydration—removal of water.

exfoliated cells—cells which have been shed from the body, often in sheets or layers.

frozen section—water content of a fresh tissue specimen is frozen to give rigidity for immediate sectioning, staining, and diagnosis; especially where cancer is suspected.

fixative—solution to prevent decomposition of tissue and to preserve it.

histology—study of tissue.

microtome—machine with a knife blade for cutting tissue in very thin slices.

mount—to place tissue sections on slides for staining and/or to cover with a cover slip for permanency.

Papanicolaou smear—called a Pap smear; a method of preparing and evaluating exfoliated cells for the diagnosis of cancer.

postmortem—autopsy.

staining—identification of tissue or cell parts by their color reaction to dyes.

In some hospitals, cytology and histology are located together in the pathology department. In other hospitals, they are separate departments. *Cytology* is concerned with cells discarded by the body while *histology* deals with tissues. Both accept tissues and cells for fixing, sectioning, staining, and mounting on slides. Technicians identify normal and abnormal cell structures including cancer during microscopic examination. A morgue in a hospital may be closely associated with the pathology department since the pathologist performs autopsies. The other departments in the laboratory are called "the clinical laboratory" or "the medical laboratory."

Types of Specimens

Disease and infection cause changes in tissues and cells. Tissues may be removed for study after death during autopsy, as surgery is performed, or during an examination by a physician. An example of a surgical specimen is an appendix removed during an appendectomy. A *biopsy* is living tissue removed from a person for microscopic examination to aid in diagnosis. Biopsies are usually obtained with a scalpel and occasionally with a needle (e.g., from a lymph node or from the bone marrow). Other specimens will arrive as smears on slides, such as Pap smears, or as fluids, such as urine, amniotic fluid, nipple discharge, cerebrospinal fluid, or other body cavity fluids. Some tissues are collected into containers with 70% alcohol.

Specimen Records

As tissue arrives in the laboratory, it is assigned a number and logged into a record book by number and patient name or added to computer records. Copies of requisitions may also be kept and filed alphabetically by patient name and the case number added.

Facilities choose their own numbering systems. The case number may include letters as part of the code used for identification. One series of letters may indicate specimen sources—S for surgery and A for autopsy. Another series of letters may indicate a facility—D for doctor's office and H for hospital. Numerals may indicate the date of collection—last two for the year and another set of two for the month. Case numbers usually change each year. In a large laboratory, numbers may change each month. In a small lab, this may not be so. With such a system, slides can be located if the date is known.

Processing Tissues

The pathologist will study large sections of tissue or whole organs in a gross examination. The physician describes conditions observed and indicates parts for study. Tissues are processed by histotechnologists and histotechnicians. Cell screening is performed by cytotechnologists. Slides are studied to determine the type and number of cells present, whether any are abnormal, and whether more testing is necessary. The size, shape, general appearance, and staining qualities of cells are observed. The cell nucleus and any cellular inclusions are examined.

The process that tissue undergoes before permanent mounting on slides includes these steps: embedding, blocking, cutting or sectioning, staining, clearing, and mounting.

Embedding is a process of placing tissue into a medium to keep it intact for processing and placement on a slide for microscopic examination. The ideal size of tissue for processing is 2 cm square and 0.4-0.5 cm thick (4-5 mm). Embedding is further divided into fixation, dehydration, clearing, and infiltration.

- *Fixation*—done with a mixture of chemicals to kill the fresh tissue, to prevent decomposition by its own enzymes, and to harden it.
- *Dehydration*—removal of water by chemicals such as alcohol or acetone.
- *Clearing*—removal of alcohol by an agent that must also be a solvent for the paraffin in the next step of the process; xylene is used as a clearing agent.
- *Infiltration*—allowing an embedding medium to penetrate the tissue for support; paraffin and collodion used in the past have been replaced by waxes and plastics.

Each tissue must be kept separate. Some of the steps in embedding can be done automatically by a machine, which takes 9 hours to process in an overnight run, or 22 hours if allowed to proceed over a weekend.

Embedding or *blocking* occurs when fixed tissue is placed in an embedding mold together with an identification tag and blocking material. Material is added to about twice the thickness of the specimen but not more than 2 mm all around the tissue. The block is chilled or hardened.

Cutting or Sectioning

The next step is *cutting* or *sectioning*. The block of tissue is fastened to a holder on a rotary microtome. The side of the block must be parallel to the knife edge along the entire surface facing the knife in order to cut a smooth ribbon. The knife edge must be kept very sharp with no nicks as these will show on the tissue on the slide under the microscope. As the microtome wheel is rotated, the knife will cut a ribbon of paraffin slices with tissue embedded. When the ribbon is 6 to 8 inches long, it is moved carefully with a brush to the surface of the water in a constant temperature water bath. The best sections are selected. A slide is slipped under the section, centered, and separated from the ribbon. Slides may have albumin on the surface as a fixative to hold the tissue. Slides are blotted and placed on a warming table for drying. Each slide must carry an identification number from the block.

Staining

The object of *staining* is to identify tissue components and cell parts by their color reaction to dyes. Hematoxylin and eosin are the dyes most often used. Hematoxylin stains the nucleus a dark blue color. Eosin is an acid stain which colors cytoplasm a rosy-red color. The color reaction occurs because the dye penetrated and caused a reaction, thus forming another substance with a certain color. A second dye may be applied, called a *counterstain*, which adds contrasting colors to bring out differences between elements in cells or tissues.

Slides are placed in slotted trays and moved by hand from staining dish to staining dish with washes between, as indicated by the procedure (see fig. 1). In a small laboratory, only one or two slides might be stained at a time. Automatic stainers, which are run by computer, are available for staining a large volume of slides.

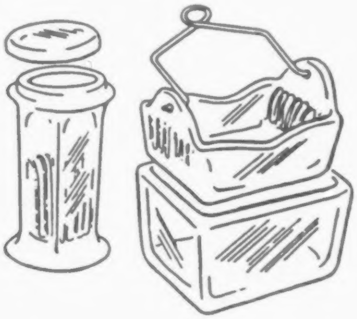


Fig. 1. Staining jars and rack

Slides can be stained using the same techniques and dyes as hematology and microbiology. These techniques include Wright's stain for blood smears, Gram's stain for bacteria, acid-fast stain for Mycobacterium tuberculosis, and Lacto-Phenol Cotton stain for fungi. Special stains and special techniques are used to bring out cellular structures. Examples are listed here:

- Peroxidase reaction—to aid in identifying cells indicating acute leukemia
- Periodic Acid-Shiff stain (PAS)—for intracellular glycogen
- Sudan Black B—stains lipids; used to detect acute leukemia
- Prussian blue stain—shows a type of iron found in red blood cells, bone marrow, the urine of patients with a hemolytic transfusion reaction, and excessive amounts in hemochromatosis.

Clearing and Mounting

Clearing is done at this point in the process to make the microscopic preparations transparent. Clearing chemicals should be solvent in the mounting medium, which is used next.

During *mounting*, a medium is applied and a cover slip added. Both the mounting medium and the cover slip must be completely transparent. The mounting reagent must form a film over the tissue which is the size of the cover glass. Excess reagent is wiped away. The cover slip must be large enough to form a margin around the tissue. The slide is allowed to dry. The label is checked for completeness.

Slides made from a specimen are marked with the case number and filed by number in sequence to correspond with the daily data log.

If a slide has a frosted end, the case number is written there with black glass marker or a lead pencil. When slides do not have a frosted end, the number is etched on the slide with a diamond point pencil.

The Papanicolaou Smear

The number of deaths due to cervical cancer has been greatly reduced by the routine use of the Papanicolaou (Pap) smear for screening. Specimens are collected by a physician from the cervix or vagina using a small spatula. The specimen is placed on a slide, labeled, put in a special container with a fixing solution, and delivered to the laboratory.

In the lab, the slide is prepared and stained for microscopic study. The cells are observed and grouped into categories to indicate normalcy, precancerous conditions, or a malignancy. The cells may also be examined to determine effects produced by hormones and diagnosis of inflammatory conditions or presence of pathogens.

The Pap smear is most often used to examine specimens from the female genital tract. This technique can be used also on the following types of specimens: duodenal drainage; gastric and nipple secretions; prostate smears; urine; sputum; and washings from the bronchi, esophagus, or digestive tract. Fluid specimens must be centrifuged. The smear is made from the sediment.

Storage of Slides

Facilities can choose methods for storage of slides (see fig. 2). Once slides have been processed, they are placed flat in cardboard trays with numbers in sequence. The trays have space for up to 20 slides. Trays may be stacked. This type of storage is temporary and is usually used during screening and evaluation processes.

For further study, slides may be placed on edge in wooden or plastic boxes. These boxes have slots providing space for 12 to 100 slides. These boxes may be stacked.

For long-term storage, the slides are placed in metal or wooden filing cabinets. Space is provided for thousands of slides placed in drawers. Slides may be placed in slots on the side or end to end with the label end up.

Safety Precautions

During the entire process, certain safety precautions must be kept in mind.

- Glove before handling body tissues and fluids.
- Patient and tissue must be identified properly.
- Tissue must carry number through process without loss or confusion.
- Small bits of tissue must be wrapped or secured while processing to prevent loss of the specimen.
- Carefully prepare ingredients for reagents, dyes, and stains according to manufacturer's directions.
- Discard solutions at date indicated for shelf life of solutions.
- Handle reagents carefully.
- Keep fingers away from knife blade in microtome.
- Check temperature often on water baths and drying oven to prevent damage to tissue during processing.
- Keep surface of water in tissue floating bath free of broken bits of tissue or embedding medium that could float over to contaminate another slide.
- Never store food or drink in the same refrigerator where tissue specimens or reagents are kept. Cross contamination can occur.
- Be certain that lids and covers are replaced on all reagents.

Precautions for Autopsies or Morticians' Services

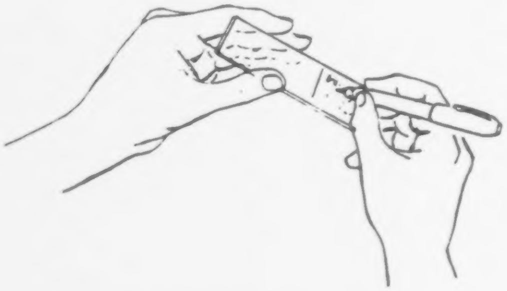
The following precautions were taken from "Recommendations for Prevention of HIV Transmission in Health-Care Settings," *Morbidity and Mortality Weekly Report*, 1987; 36 (suppl. no. 25), p. A6-54, published by the Centers for Disease Control, U.S. Department of Health and Human Services, Atlanta, Georgia 30333.

In addition to Universal Blood and Body Fluid Precautions, the following precautions should be used by persons performing postmortem procedures:

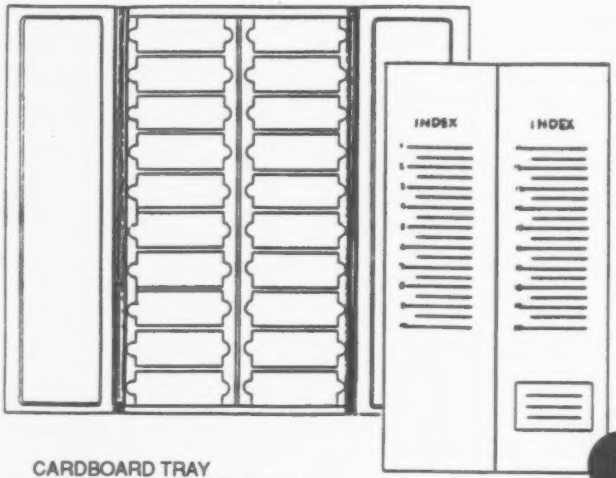
1. All persons performing or assisting in postmortem procedures should wear gloves, masks, protective eyewear, gowns, and waterproof aprons.
2. Instruments and surfaces contaminated during postmortem procedures should be decontaminated with an appropriate chemical germicide.

Summary

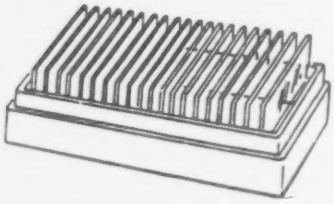
Tissues are accepted in histology/cytology for fixing, sectioning, staining, and mounting on slides. All specimens and slides must bear an identification which follows through processing and diagnosis into storage. Workers are cautioned on safety conditions unique to the pathology laboratory.



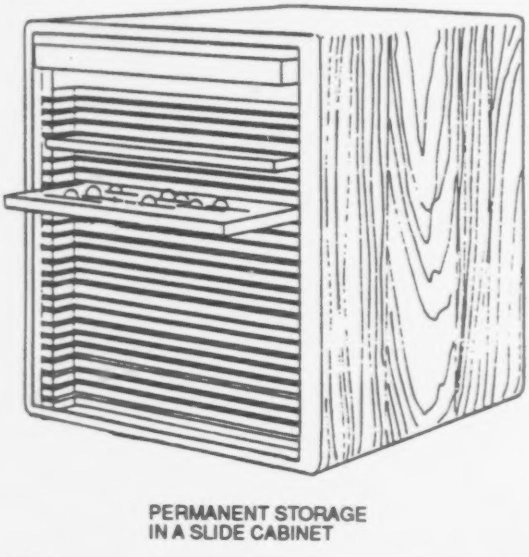
MARKING SLIDE FOR IDENTIFICATION



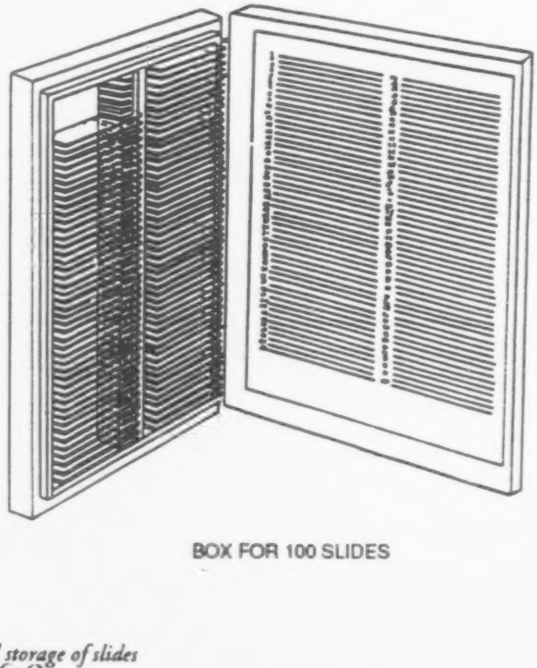
CARDBOARD TRAY
FOR TEMPORARY STORAGE



SMALL PLASTIC STORAGE BOX



PERMANENT STORAGE
IN A SLIDE CABINET



BOX FOR 100 SLIDES

Fig. 2. *Identification and storage of slides*

Medical Laboratory Abbreviations

ACD—acidified citrate-dextrose
AIDS—acquired immunodeficiency syndrome
ALT—alanine aminotransaminase
ANA—antinuclear antibody
APTT—activated partial prothrombin time
ARC—AIDS-related complex
ASO titer—antistreptolysin O titer
AST—aspartate aminotransaminase

BSP—bromsulfalein
BUN—blood urea nitrogen

CBC—complete blood count
CDC—Centers for Disease Control
CSF—cerebrospinal fluid

diff—differential white count

EIA—enzyme immunoassay
ELISA—enzyme-linked immunosorbent assay
ESR—erythrocyte sedimentation rate

FUO—fever of unknown origin

GTT—glucose tolerance test

Hb or Hgb—hemoglobin
HCG—human chorionic gonadotropin
Hct—hematocrit
HIV—human immunodeficiency virus
hpf—high-power field

LDH—lactic dehydrogenase
LDL—low-density lipoprotein cholesterol
lpf—low-power field

MCH—mean corpuscular hemoglobin
MCHC—mean corpuscular hemoglobin concentration
MCV—mean corpuscular volume
MT—medical technologist

NFPA—National Fire Protection Association
nm—nanometers

OD—optical density
OSHA—Occupational Safety and ARC—Health Act

plt—platelet count
POL—physician's office laboratory
PT—prothrombin time

QA—quality assurance
QC—quality control

RBC—red blood cell or count
retic—reticulocyte count
rpm—revolutions per minute
RPR—rapid plasma reagin test

SGOT—serum glutamic oxalacetic transaminase
SGPT—serum glutamic pyruvic transaminase
sp gr—specific gravity
staph—staphylococcus bacteria
strep—streptococcus bacteria

TC—to contain
TD—to deliver

VDRL—veneral disease research laboratory

WBC—white bood cell or count

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ASSISTING IN THE MEDICAL LABORATORY

Student Learning Activities

Assisting in the Medical Laboratory

Student Learning Activities

By

Helena J. Fair

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COMPLIANCE STATEMENT

TITLE VI, CIVIL RIGHTS ACT OF 1964; THE MODIFIED COURT ORDER, CIVIL ACTION 5281, FEDERAL DISTRICT COURT, EASTERN DISTRICT OF TEXAS, TYLER DIVISION

Reviews of local education agencies pertaining to compliance with Title VI Civil Rights Act of 1964 and with specific requirements of the Modified Court Order, Civil Action No. 5281, Federal District Court, Eastern District of Texas, Tyler Division are conducted periodically by staff representatives of the Texas Education Agency. These reviews cover at least the following policies and practices:

(1) acceptance policies on student transfers from other school districts;

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(3) nondiscrimination in extracurricular activities and the use of school facilities;

(4) nondiscriminatory practices in the hiring, assigning, promoting, paying, demoting, reassigning, or dismissing of faculty and staff members who work with children;

(5) enrollment and assignment of students without discrimination on the basis of race, color, or national origin;

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Notes for the Student

Assisting in the Medical Laboratory is designed for use by students investigating the activities within the medical laboratory in a hospital, clinic, or physician's office. The material is designed to relate training experience to information studied in the classroom. Information is provided which is current and relevant. The material is designed for individualized study and is competency-based, with educational outcomes stated for each unit. *Assisting in the Medical Laboratory* is made up of three separate publications.

- The **Student's Manual** contains educational objectives, terms and definitions, and information relating to current practices. Topics addressed are the organization, personnel, and type of equipment used in laboratories. The collection and processing of specimens is described. Techniques, both manual and automated, are described for the following departments: urinalysis, hematology, serology, microbiology, chemistry, the blood bank, and histology/cytology. Safety and quality control are emphasized.
- The **Student Learning Activities** are individualized and designed to encourage the student to carefully read and study the information presented. Each unit has a section for those students desiring more advanced study. Instructors are encouraged to provide one set of activities for each student to facilitate learning.
- The **Instructor's Guide** has a list of essential elements addressed by the units of instruction. A basic test is provided for each unit. Instructors are encouraged to enlarge these. Answers to tests and learning activities are also provided, and a competency chart is included.

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Portions of this book are adapted from two EIMC publications: *Medical Laboratory Assistant* (1981), by Sara Barnett, and *Medical Laboratory Services* (1981), by Catherine Williams.

Objective: To use terms relating to safety appropriately

Directions: From the list at the beginning of the unit, select the word which will convey the appropriate meaning to the sentence. Write it in the blank.

1. Clothes which are easily set on fire are called ______________________.
2. When an unusual event occurs, such as a patient falling, a special report is prepared, called an ______________________ .
3. Loud speakers in hospitals use special terms to signal emergencies. These are called ______________________ .
4. One place to get information about the safety regulations required for medical laboratories is from the public health agency called ______________________ and headquartered in Atlanta, Georgia.
5. The initials for this agency are ____________ .
6. Ice on roads is a dangerous situation which is called a ______________________
7. Strong acids are called ______________________ because they can react with certain types of metal caps to destroy them.
8. Contaminated materials must be burned to ashes or ______________________
9. This unit presents information to keep us safe from danger or injury. Thus, it is a unit on ______________ .
10. A fire extinguisher is used to ______________________ a fire.
11. All safety signs should be ______________________ .
12. Hazard identification symbols were developed by the ______________________ .
13. The abbreviation for this group is __________ .
14. All employees must conform to ____________ safety and health standards.
15. This abbreviation stands for ______________________ .

Objective: To identify safety practices in the laboratory and procedures for reporting accidents

Directions: Answer the following questions briefly.

1. List two agencies which help to ensure safe working conditions in medical laboratories.
2. Who is responsible for enforcing OSHA's regulations?
3. Why are "codes" used in hospitals?
4. Decide which code might be called during the following situations:
    a. bomb threat
    b. a nearby train wreck
    c. a visitor who has a heart attack
    d. a fire
5. Besides a fire blanket, cite three additional pieces of equipment which should be provided for worker safety in labs.
6. Give the location of the following items in the laboratory where you are assigned:
    a. safety shower
    b. eyewash station
    c. hoods
7. Describe a worker clothed properly for routine blood work in a laboratory.
8. List additional gear which may be required for certain procedures.
9. Explain (a) where an incident report is filed, and (b) what it must contain.
10. State the most important safety precaution to keep in mind when collecting specimens.
11. State precautions to keep in mind when using reagents.

Learning Activity 3
Fire Safety

UNIT 1
Safety in the Laboratory

Objective: To explain procedures for fire prevention, control, and reporting

Directions: Complete the first questions using information from the place where you are assigned. Answer the other questions using Unit 1.

	Location of Two Extinguishers	Type of Extinguisher	Type of Fire Used On
1.	____________	____________	____________
2.	____________	____________	____________

3. Location of fire doors____________________________________

4. Location of emergency exits______________________________

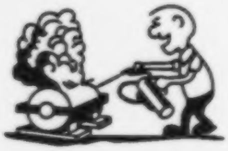
5. Location of fire blanket__________________________________

6. Describe the (a) purpose and (b) use of a fire blanket.

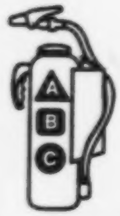
7. What public address system code is used to indicate a fire?

8. To whom do you report a fire in the laboratory?

9. Look at the picture and determine
   (a) the class of fire and
   (b) what types of extinguishers could be used on the fire.



10. Look at the picture and determine
   (a) what type of extinguisher is shown and
   (b) the types of fires on which this could be used.



11. The geometric shapes on the fire extinguishers are colored and are in different shapes. Explain what colors and what shapes are used for the following classes of fires. List flammable materials included in each group.

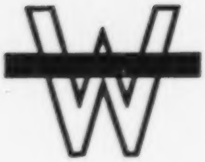
   a. Class A

   b. Class B

   c. Class C

12. (a) If this symbol were present on a reagent bottle, what would this mean?

(b) What types of fire extinguishers might you select for use if this reagent ever ignited?

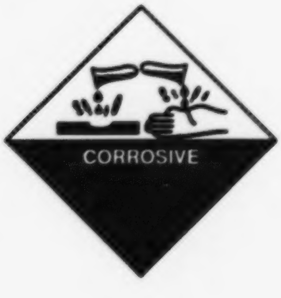


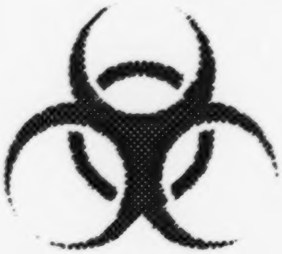
Learning Activity 4
Contaminated Materials

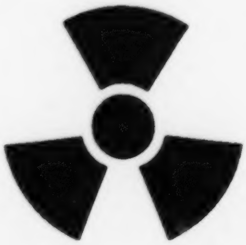
Objective: To explain how to dispose of contaminated materials safely

Directions: Answer the following questions completely.

1. Which agency developed and mandates use of Universal Blood and Body Fluid Precautions?
2. List body fluids covered by these precautions.
3. Can you think of any body fluids which are normally considered sterile?
4. Describe how to dispose of a sputum specimen.
5. Describe how spills of contaminated matter are disinfected.
6. Explain how to dispose of contaminated disposable equipment.
7. What happens to sharps after use?
8. Outline the procedure to follow each day as you prepare to leave the laboratory.
9. Which health care workers should receive hepatitis B vaccine?
10. Who pays for the hepatitis B vaccine?
11. Look at figure 3, "Donning and Removing Gloves." Tell why the fingers are inside the cuff in drawing B.
12. Look at the symbols below. Select the one found on biohazards.







Objective: To dispose of contaminated materials and equipment using universal precautions

Directions: Use the material in figure 5 to answer the following questions.

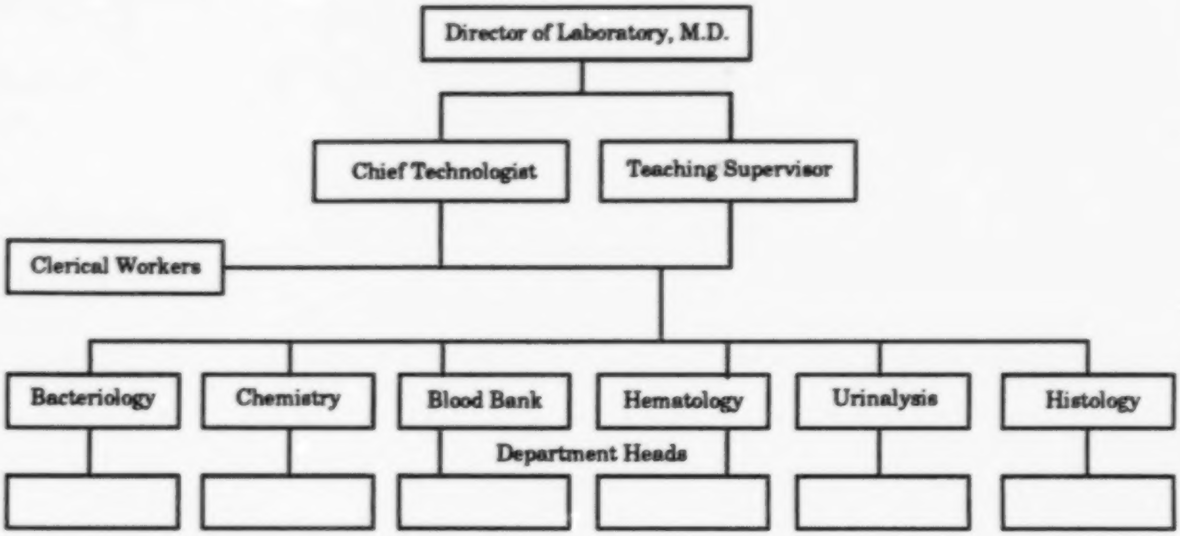
1. Define the term *health care worker*.
2. Describe the container recommended for transport of specimens.
3. Define *sonicating*.
4. On what occasions is pipetting by mouth allowed?
5. On what occasions are needles and syringes used?
6. What procedure is followed for disposal of contaminated testing materials?
7. If laboratory equipment needs repairs, what procedure should be followed before the repairs are undertaken?
8. Describe the procedure recommended after laboratory activities are completed.

Learning Activity 1
Personnel of a Laboratory

UNIT 2
Organization of the Laboratory

Objective: To identify medical laboratory personnel, their credentialing, and their organization for work in the laboratory

Directions: To the chart below, add the names of the people in the lab where you are assigned. Below the level of department head, draw a short line down and list the names of everyone in the department. Add the proper abbreviation (M.T., M.L.T., etc.) after each name. List and indicate laboratory assistants.


Director of Laboratory, M.D.
Chief Technologist
Teaching Supervisor
Clerical Workers
Bacteriology
Chemistry
Blood Bank
Hematology
Urinalysis
Histology
Department Heads

Learning Activity 2
Levels of Education and Responsibility

Objective: To identify types of degrees and levels of education for laboratory workers

Directions: Match the following terms to the definitions by writing the letter in the blank:

____ 1. list of qualified individuals kept on an official record

____ 2. highest degree awarded by universities

____ 3. degree awarded after completion of 2–3 years of study beyond a bachelor's degree

____ 4. awarded by a college or university after completion of undergraduate studies

____ 5. awarded by a junior or community college after completion of prescribed two-year study

____ 6. issuance of a certificate by a regulating professional organization

____ 7. permission to practice given by state or national board to person with a proved competency

a. associate degree

b. baccalaureate degree

c. certification

d. doctorate

e. licensure

f. master's degree

g. registry

Objective: To identify laboratory personnel

Directions: Use the sections on "Laboratory Personnel" and "Physicians" to identify the laboratory worker.

1. Medical specialist who examines cells and tissues to aid in diagnosing and treating
2. Medical specialist who treats illness of adults nonsurgically and sometimes heads a laboratory
3. Laboratory worker who is trained on the job
4. Worker who draws blood samples
5. Individual who has completed two years of laboratory training at a junior or community college and passed an examination
6. Worker trained to prepare and study slides of human cells
7. Worker trained for two years to prepare tissues for microscopic examination
8. Person with bachelor's degree and one year of laboratory training who performs complex laboratory procedures
9. Worker with a baccalaureate degree who prepares body tissue for microscopic study
10. Medical specialist who performs autopsies
11. Type of laboratory worker who supervises the performance of other workers
12. Medical specialist who supervises laboratory workers

Learning Activity 4
Work Areas within the Laboratory

UNIT 2
Organization of the Laboratory

Objective: To differentiate the types of work performed in various areas of the laboratory

Directions: Listed below are laboratory tasks. Indicate in which department each would be performed. Write the letter indicating a department in the blank. Use locations indicated in the Student's Manual, as some of the procedures may vary in location.

____ 1. determination of blood sugar

____ 2. determining presence of glucose in urine

____ 3. identification of abnormal cells of Pap smear

____ 4. drawing of blood samples

____ 5. determining presence of worms in feces

____ 6. examination of urine for blood

____ 7. performing a pregnancy test

____ 8. count of number of WBC in a blood sample

____ 9. carrying out blood coagulation studies

____ 10. identification of bacteria causing an infection

____ 11. drawing donor blood

____ 12. preparing tissues for staining

____ 13. isolating medications for stopping the growth of specific bacteria

____ 14. cross-matching blood for patient use

____ 15. determining whether spinal fluid has bacteria in it

____ 16. determining amount of calcium in blood

____ 17. a protein determination on spinal fluid

____ 18. testing for syphilis

a. blood bank
b. chemistry
c. cytology
d. hematology
e. histology
f. microbiology
g. parasitology
h. phlebotomy
i. serology
j. urinalysis

____ 19. checking for cause of a transfusion reaction

____ 20. identifying abnormal types of RBCs on a blood smear

Learning Activity 5
Computers in the Laboratory

Objective: To describe the use of computers in the medical laboratory

Directions: Answer the following questions taken from the last section of Unit 2.

1. What is a computer?
2. What is a terminal?
3. What is hard copy?
4. State the two major divisions of the medical information system.
5. Explain the role of computers in communication between departments within a hospital.
6. Explain the uses of computers within the laboratory itself.
7. What types of computers are used in the laboratory where you are assigned?
8. Give the location of a computer for networking with departments in the facility.
9. List locations and uses of computers within the laboratory.

Learning Activity 1
Terms Relating to the Processing of Specimens

UNIT 3
Collecting and Processing Specimens

Objective: To use terms relating to the collection and processing of specimens appropriately

Directions: Use the terms at the first of the unit to supply words to complete the sentences in the following story.

Amy Visits the Laboratory

Amy was very (1) ______ about going to the laboratory to have a blood sample taken. Her mother cautioned her not to act (2) ________ to the laboratory worker. Her mother said that the phlebotomist would try to establish a good (3) _____ with her. Her mother explained that drawing blood was easier than having body tissue removed, a process called a (4) _____________, or collecting a urine specimen with a (5) ____________.

At the clinic the phlebotomist explained to Amy that he would use a small, sharp-pointed instrument called a (6) ________ to stick her finger. If he drew blood from her arm, called (7) _____ , he would have to use a syringe and a needle with a sharp (8) __________. The phlebotomist was careful in collecting blood to prevent (9) _________ (the rupture of RBC with the release of hemoglobin).

Amy's blood was taken to the laboratory for centrifuging. It separated into layers, with the heavier parts at the bottom and the (10) __________ at the top. If there is an anticoagulant in the collecting tube, the fluid is called (11) __________. An anticoagulant is a (12) __________________. Clotting is also called (13) ________ . If no anticoagulant is present, the fluid is called (14) _____ . If either the plasma or the serum has a white, cloudy appearance due to excess fat, the liquid portion is said to be (15) _________ .

Learning Activity 2
Interacting with Patients

UNIT 3
Collecting and Processing Specimens

Objective: To receive, dismiss, and interact with patients in a professional manner

Directions: Using the sections from "Developing Good Patient Relationships" through "Professionalism," develop a list of "Do's" and "Don'ts" for patient relationships.

Things to Do for Good Rapport	*Things to Avoid with Patients*

Learning Activity 3
Collection of Specimens

UNIT 3
Collecting and Processing Specimens

Objective: To describe types of specimens received in the laboratory, their collection, and their processing

Directions: Answer the following questions:

1. What method of blood collection is used if more than a few drops are required?
2. List ways to identify a patient.
3. Where is the most satisfactory area for the collection of venous blood?
4. Why must you remove the needle from the syringe before putting blood in tubes?
5. For what reason are vacuum tubes used in collecting blood samples?
6. List four anticoagulants found in blood-collecting tubes.
7. Why are stoppers on tubes different colors?
8. List three tips to remember to prevent hemolysis of blood.
9. From what body areas other than fingertips may capillary blood be collected?
10. Name four items that the patient must fully understand when you are giving instructions for the collection of feces.
11. List five types of tests that can be done on feces.
12. What is routine urinalysis?
13. Name two other types of urinalyses.
14. State the purposes for collecting specimens.
15. Name five methods by which a specimen may be obtained.
16. What does "logging" specimens mean?
17. Describe methods used to identify specimens.
18. What does "processing" a specimen mean?
19. What is the general rule for processing materials?

20. Why are specimens sent to other laboratories for testing?

21. In the facility where you are assigned, (a) what method of filing is used (name, number, etc.) and (b) where and how are records stored?

Learning Activity 4
Collection of Blood Samples

UNIT 3
Collecting and Processing Specimens

Objective: To differentiate between venipuncture and capillary blood collection

Directions: Below are listed steps for collecting blood by venipuncture and by sticking a finger with a lancet. Indicate the correct order by placing a number in each blank.

Venipuncture Using a Syringe and Needle

A. Apply firm pressure, and flex arm until bleeding stops. ____A.

B. Place a tourniquet above the site, tying so that it can be released with one hand. ____B.

C. Insert the needle cleanly and rapidly with the bevel pointing upward. ____C.

D. Place a sponge over the puncture site, but do not apply pressure until the needle is withdrawn from the vein. ____D.

E. Aspirate blood. ____E.

F. Cleanse with a disinfectant sponge. ____F.

Collecting Capillary Blood from a Finger

A. Prepare blood smears if required, and apply pressure with a sponge. ____A.

B. Grasp finger across the middle or first joint, and puncture skin with lancet. ____B.

C. Cleanse with a disinfectant sponge, and wipe with a dry sponge. ____C.

D. Select the site to puncture. ____D.

E. Wipe off first drop of blood, and collect with a continuous flow into containers. ____E.

List the precautions to observe before collecting specimens—

a. for yourself

b. for the patient

Learning Activity 1
Terminology Relating to Equipment

Objective: To recognize terms associated with equipment

Directions: Use section "Terms and Definitions" to answer the following questions:

1. A cylindrical glass tube for measuring and transferring liquids is the 1. ____________
2. To make free of all living organisms is to 2. ____________
3. Apparatus used to sterilize by steam under pressure is the 3. ____________
4. An automatic device to control temperature is a 4. ____________
5. A mark on a piece of glassware to indicate the amount it will measure is a 5. ____________
6. A transfer pipette that measures a single amount is a 6. ____________
7. The crescent-shaped curvature found on the upper surface of a liquid as it is measured is a 7. ____________
8. A machine to spin at high speed for separating materials of different densities is a 8. ____________
9. Pipette to measure various volumes within a given amount is a 9. ____________
10. Straight-sided tube with a base used for measuring the volume of liquids 10. ____________

Directions: Give the meaning for the following abbreviations:

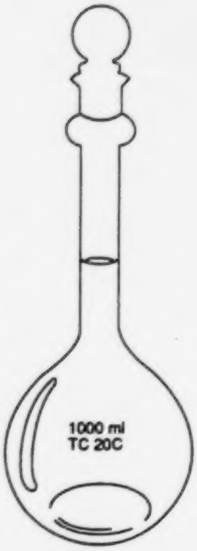
11. rpm
12. TC
13. TD

Learning Activity 2
Identification of Equipment

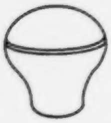
UNIT 4
Laboratory Equipment

Objective: To identify laboratory equipment by name

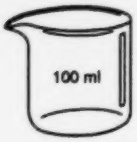
Directions: Write the names of the following pieces of equipment in the proper blanks.


1000 ml
TC 20C

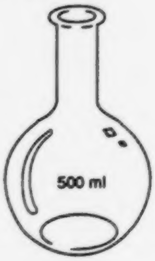
3. ______________



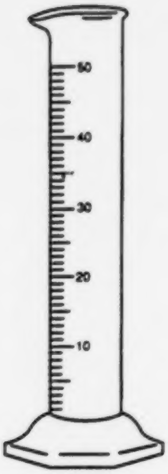
1. ______________



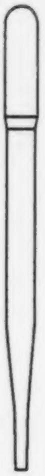
2. ______________



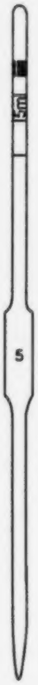
4. ______________


50
40
30
20
10

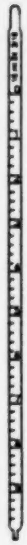
5. ______________



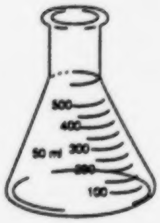
6. ______________



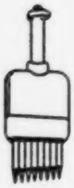
7. ______________



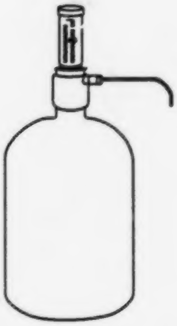
8. ______________


500
400
50 ml
300
200
100

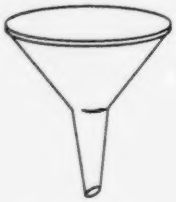
9. ______________



10. ______________



11. ______________



12. ______________

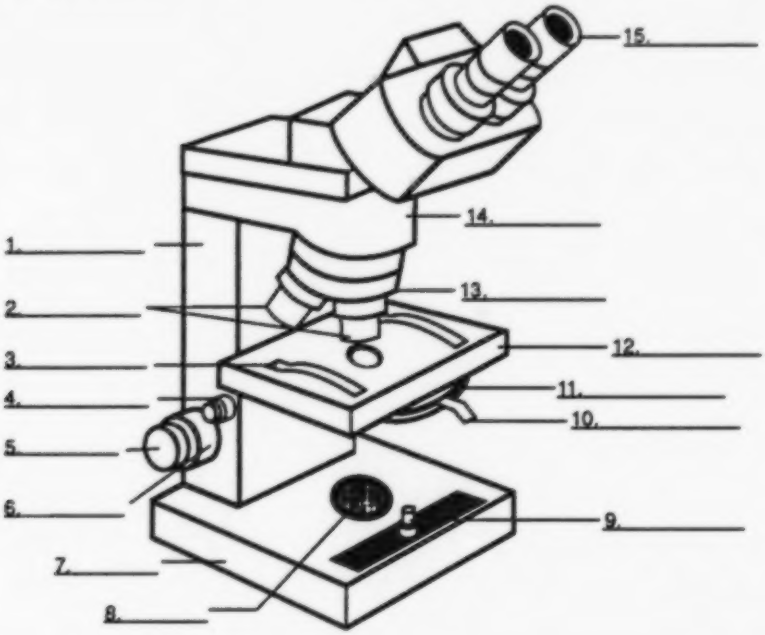
Objective: To describe how equipment is stored and used

Direction: Answer the following questions:

1. Cite two factors for consideration in the storage of glassware and equipment.
2. Describe how the meniscus is used in reading the volumes of liquids in calibrated glassware.
3. Which type of flask would you select for greatest accuracy?
4. Describe a beaker. Can it be used for accurate measurement?
5. List two purposes of pipettes, and give an example of the type of pipette used for each purpose.
6. Describe the process for using a pipette.
7. Explain how centrifuges are used to separate substances.
8. For what are centrifuges used in the laboratory?
9. Name three special types of centrifuges.
10. Explain why it is necessary to perform routine checks of centrifugation.
11. Name three devices that can be used to calibrate centrifuges.
12. Name two pieces of equipment that may be used for sterilization in the laboratory.
13. List two constant-temperature appliances in the laboratory.
14. Survey the laboratory where you are assigned. List all of the constant-temperature appliances in this laboratory.

Objective: To identify parts and use of a microscope

Directions: Label the parts of the microscope shown in the drawing and answer the questions.



16. Differentiate between a monocular and a binocular microscope.

17. Which type of microscope gives the greatest magnification?

18. Explain the difference between the coarse adjustment wheel and the fine adjustment wheel.

19. Why should the movement of the barrel be watched when you are using the coarse adjustment wheel?

20. List two devices used to provide light on microscope slides.

21. If an eyepiece has 10X marked on it, what does this mean?

22. If you view a slide through a 10X eyepiece and a 20X objective, how great is the magnification of the object on the slide?

23. What is used to clean (a) the low-power objective on a microscope and (b) the oil-immersion objective?

24. Describe the correct method for carrying a microscope.

Learning Activity 1
Terms Relating to Urinalysis

Objective: To associate terms with procedures in urinalysis

Directions: Use "Terms and Definitions" to answer the following questions:

1. Select four terms which refer to conditions affecting the urinary system. List and define these.
   a.

   b.

   c.

   d.

2. Differentiate between *microscopic* and *macroscopic*.

3. Look up the meaning of the word *micturate*. Locate two terms with the same meaning and write these two words.
   a. b.

4. Some terms apply to the microscopic examination of urinary sediment.

   a. Define *sediment*.

   b. Locate a term used in the preparation of the sediment. List and define it.

   c. Select four terms which are used in regard to the microscopic examination of urinary sediment. List and define these.

5. Some casts in sediment are described as *convoluted*. What does this mean?

6. To what is urine compared when determining its *specific gravity*?

7. Differentiate between *quantitative* tests and *qualitative* tests.

Learning Activity 2
Scrambled Terms

UNIT 5
Urinalysis

Objective: To recognize terminology relating to urinalysis

Directions: Unscramble the following letters to form words. These are from "Terms and Definitions." Make a related word from the circled letters.

1. S C I Ⓘ P F E C T R G Ⓨ V A I
2. A A E I T T Ⓤ Q N V I
3. V D Ⓘ O
4. D T M N Ⓢ E E I
5. S S C Ⓐ T
6. N N T T P R Ⓢ U E A A
7. C C T Ⓛ O U
8. O O C C C I I Ⓡ M P S
9. T C D Ⓝ A E

1. ______________________________

2. ______________________________

3. ______________________________

4. ______________________________

5. ______________________________

6. ______________________________

7. ______________________________

8. ______________________________

9. ______________________________

10. ______________________________

Objective: To obtain a specimen and conduct a macroscopic urinalysis

Directions: Answer the following questions:

1. Who is responsible for the collection of routine urine specimens in (a) the hospital and (b) the clinic?
2. What two kinds of specimens are normally processed as "routine urines"?
3. Why should examinations be carried out on fresh urine specimens?
4. List four items of information that should be included on a urine specimen in a clinic laboratory.
5. In a hospital setting, what information must be added to this?
6. If a patient appeared at the clinic laboratory with a urine specimen in a tobacco can, what should the medical worker say or do?
7. Name two items reported in the macroscopic examination of urine.
8. If a urine specimen were received showing the following colors, what would you suspect was the cause?
    a. blue
    b. colorless
    c. dark brown
    d. yellow
9. What is meant by the *character* of urine?
10. How is character described?
11. Name three things that cause cloudiness in urine.
12. Give the normal range of specific gravity for urine.
13. In general, what causes specific gravity to drop?
14. Name two instruments used to determine specific gravity.

15. Which instrument should be used for a small sample of urine?

16. Define *pH*, and give the normal range for urine pH.

17. How is urine pH determined?

18. How is chemical content of urine determined?

19. Pathological conditions are indicated by the presence of certain substances. List eight substances whose presence in urine is considered abnormal.

20. Describe the general procedure for using reagent strips.

Objective: To conduct a microscopic examination of urine

Directions: Answer the following questions:

1. Describe how you would prepare a urine sample for a microscopic examination.
2. Why would you use Sternheimer or crystal-violet safranin stain?
3. List at least six errors to avoid in performing microscopic examinations.
4. Explain (a) what the abbreviations *lpf* and *hpf* mean; (b) how they are determined; and (c) examples of three structures which may be reported in this manner.
5. What type of reporting is used by the institution where you are assigned? (words, average numbers, or pluses)
6. List seven kinds of cellular structures that may be found microscopically in urine samples. Sketch any three you have listed.
7. What is *Trichomonas?* Is it normally found in urine?
8. Describe or sketch each of the following kinds of casts.
   a. hyaline cast
   b. finely granular cast
   c. coarsely granular cast
   d. red cell cast
   e. white cell cast
   f. fatty cast
   g. waxy cast
9. Name two structures that may be mistaken for casts.
10. List seven kinds of crystals that may be found in acid urine samples.
11. List five kinds of crystals that may be found in alkaline urine samples.
12. Name two structures that may be mistaken for crystals.

Objective: To complete a urinalysis report manually

Directions: Given the information, completely fill in the request form below.

1. Mack Jones, age 16 months, patient number 220690, room 489, chart number 1637259, Dr. Sally Smith; ward clerk SB filled out request slip at 7:15 A.M. on present date; voided urine was collected at 8 A.M. by nurse aide BS and transported to lab for routine.

2. Jayne Pinkston performed the urinalysis and did a microscopic examination on the centrifuged sediment with these findings: The urine was yellow and appeared partly cloudy. It had a specific gravity of 1.026 with a 6.0 pH. The Labstix indicated 100 mg % albumin (protein), and the blood was positive. There was no sugar, acetone, bile, or phenylpyruvic acid. Microscopically, she observed calcium oxalate crystals, 4–8 finely granular casts and 1–3 hyaline casts per low-power field, and 6–10 red cells per high-power field. Fee was $8.00.

3. What would have been different if Mack Jones had been 16 years of age rather than 16 months?

10 URINALYSIS

Name	**Age**	CHARGE	PHYSICIAN ORDERING TEST	
Patient Number	**Sex**		TO BE DONE	
Chart Number		**Room**	TIME-DATE COLLECTED-INITIALS	
REQUESTED BY INITIAL	TIME ☐ AM ☐ PM	DATE	REPORT DATE & TECH.	

☐ VOIDED
☐ CATH.
☐ URETER
L ☐ R ☐
☐ RT. URINALYSIS
☐ MICROSCOPIC ONLY
☐ OTHER

(2) COLOR: (3) CHARACTER: (4) PH: (5) SP. GR. 1.0

6 ☐ ALBUMIN ______
7 ☐ SUGAR ______
8 ☐ ACETONE ______
9 ☐ OCCULT BLOOD ______
10 ☐ BILE ______
11 ☐ PHENYLPYRUVIC ACID ______

(12) WBC ______ /HPF
(13) RBC ______ /HPF (21) AMOR. URATES ______ /HPF
(14) EP. CELLS ______ (22) AMOR. PHOSP ______ /HPF
(15) BACTERIA ______ (23) YEAST ______ /HPF
(16) CRYSTALS ______
(17) MUCUS ______ (24) TRICH. ______ /HPF
(18) HYALINE CASTS ______ /LPF (25) SPERM ______ /HPF
(19) GRANULAR CASTS ______ /LPF OTHER ______
(20) OTHER CASTS ______ /LPF ______

BEST COPY AVAILABLE

Objective: To complete a urinalysis report by computer printout

Directions: Below is a form for urinalysis results which would be printed outwhen reporting by computer. Priority is *Today,* as it is not *Priority 1,* meaning STAT, or at once. Indicate neg (negative) if substance was not present in chemical analysis. Fill out form using the same information given for the manual form.

Name:
Patient number:
Med. Rec. No.:
Doctor:
Diagnosis:

Loc:
Age:
Date:
Time:

Room:
Sex:

URINALYSIS

Date:
Time:
Priority:

	Normal	*Units*
Glucose	negative	mg/dl
Bilirubin	negative	
Ketone	negative	mg/dl
Spec. gr.	<1.030	
Blood	negative	
pH	5.0 to 8.8	
Protein	negative	mg/dl
Urobilinogen	0.2 - 1.0	EU/dl
Nitrite	negative	
Leukocytes	negative	
Phenylpyruvic acid	negative	

Microscopic

Date:
Time:
Priority:

	Normal	*Units*
Mucous		
Crystals		
Bacteria		
Epi cells		
WBC		/hpf
RBC		/hpf
Tric		/hpf
Sperm		/hpf
Casts		/lpf

Learning Activity 1
Terms Relating to Hematology

Objective: To recognize and use terms relating to hematology

Directions: Identify the meanings of the following abbreviations; then answer the questions.

1. CBC
2. ESR
3. HbS
4. MCH
5. MCHC
6. MCV
7. MPV
8. RBC
9. WBC
10. µ

Directions: Select, list, and identify six terms relating to types of blood cells.

11.

12.

13.

14.

15.

16.

17. Differentiate between heterozygous and homozygous gene conditions.
18. What causes fluids to rise in narrow tubes?

19. List the names of two devices used for measurements in hematology.

20. What is hemoglobin, and where is it found?

Objective: To explain the significance of the tests included in a CBC and other tests of blood components

Directions: Answer the following questions from the first part of the unit:

1. Define *hematology*.
2. List the separate tests included in a CBC.
3. What does a high WBC usually indicate?
4. What does it mean if a patient is said to be anemic?
5. What does a hematocrit measure?
6. What is the function of the thrombocytes (platelets)?
7. Complete the following paragraph, which describes cell counting using a Coulter instrument.

   As cells pass through the aperture and displace an equal volume of (a)__________, the electrical resistance in the path of the (b)________________ changes. This results in corresponding current and (c)____________________ changes. The amount of this change is directly proportional to the (d)_________ of the cell.
8. Why must Coulter counts be corrected with a Coincidence Correction Chart?
9. Sedimentation rates (ESR), in general, correlate to inflammatory processes. How can the ESR best be utilized?
10. Sedimentation rates must be corrected unless a hematocrit is (a)___________ for a female or (b)____________ for a male.
11. How are reticulocytes distinguished?
12. How many RBCs must be counted when a reticulocyte count is performed?
13. If 22 "retics" are counted, how is this reported?
14. When performing a Wright's stain on blood smears, how do you mix the stain and the buffer that are on the slide?
15. How are leukocytes reported in differential counts?

16. What are the two general types of leukocytes?

17. Distinguish each of the following leukocytes:

    a. eosinophils

    b. basophils

    c. band cells

    d. neutrophils

    e. lymphocytes

    f. monocytes

18. Besides reporting the number and kind of leukocytes present when reporting a differential cell count, what else must be commented upon?

19. The following items are frequently used to describe erythrocytes. To what does each refer?

    a. anisocytosis

    b. poikilocytosis

    c. polychromatophilia

20. Name two methods employed for platelet counts.

21. Identify the following by stating in which test each is employed.

    a. capillary tubes

    b. curvette

    c. hemocytometer

    d. hemoglobinometer

    e. methylene blue solution

    f. Sicklequik tubes

    g. Wintrobe tube

    h. Wright's stain

Objective: To collect and complete tests for coagulation studies

Directions: Use the last part of the unit to answer the following questions.

1. What ratio of blood to anticoagulant is desired for coagulation studies?

2 Name three anticoagulants.

3. Identify the order (by name and/or color of top) in which tubes of blood are collected if more than one kind of tube is necessary to perform tests that are ordered for coagulation studies.

4. Why is it necessary to mix citrate (blue-topped tubes) immediately?

5. For what are tubes for coagulation studies checked? Why?

6. Name four variables that must be closely monitored to obtain optimum coagulation results.

7. What is meant by bleeding time?

8. Name a method for determining clotting time.

9. Briefly, what does coagulation consist of?

10. a. Where is thromboplastin normally located?

    b. What is the purpose of thromboplastin?

    c. How does blood come in contact with thromboplastin?

11. What does the prothrombin time measure?

12. Name the condition in which the protime is most useful.

13. a. State the normal range for a prothrombin time.

    b. State the therapeutic range of prothrombin times.

14. a. Explain what may occur if the prothrombin time increases to over 40 seconds.

    b. Explain what can happen when the protime is allowed to drop back into the normal range during treatment.

15. Identify the single most useful procedure for routine screening of coagulation disorders.

16. What does activated partial thromboplastin time (APTT) measure?

Learning Activity 4
Scrambled Terms

UNIT 6
Hematology

Objective: To recognize words associated with hematology

Directions: Unscramble the following letters to form words. These are from "Terms and Definitions." Make a related word from the circled letters.

1. BLNGⒽMOOIE 1. ____________

2. SⒺR 2. ____________

3. ZGHⓂYOOOUS 3. ____________

4. RCGNLYTEⒶOU 4. ____________

5. TLLⓉPEEA 5. ____________

6. TRTHCMⓄAEI 6. ____________

7. YIAAPCLRⓁCTNIAⓄ 7. ____________

8. TZYⒼHRSOEOEU 8. ____________

9. CTTRLⓎCEIOEU 9. ____________

10. ____________

Objective: To calculate blood indices

Directions: Given the following information, calculate the MCV, MCH, and MCHC for each.

1. Hgb = 12; Hct = 35; RBC = 4,000,000
2. Hgb = 10; Hct = 31; RBC = 3,600,000
3. Hgb = 16; Hct = 48; RBC = 5,000,000
4. Hgb = 14; Hct = 41; RBC = 4,800,000
5. Hgb = 11; Hct = 36; RBC = 3,000,000

Learning Activity 6
Hematology Report Form

Objective: To complete a hematology report form for both manual and automated methods

Directions: Given the following information, complete the hematology form below. (Refer to figure 1 in Unit 5 for an example.)

Kara Fair, age 15, patient number 941492, was seen in the Emergency Room by Dr. Nathan Williams, who ordered a CBC (which at this hospital includes Hct, Hgb, WBC, RBC, Indices, and Diff), platelet count, and sed rate to be done STAT on 4-14 of this year. Brenda Paxton phoned the lab with the request at 1:45 P.M. D. D. Morris collected the blood in the ER at 2:00 P.M. Melba Wood was the medical technologist who performed the tests on the blood, with these results: 15,300 WBC; 4,230,000 RBC; 10.0 Hgb; 39% Hct. The Diff showed slight anisocytosis with a moderate number of microcytes; slight poikilocytosis; and a few target cells with 1% basophils, 4% eosinophils, 16% lymphocytes, 4% monocytes, 10% bands, and 65% segmented neutrophils. There were 225,000 platelets, and the corrected ESR was 20. The charge was $25.00. Figure the indices.

NAME CHART NUMBER	☐ CBC ☐ HCT ☐ HGB ☐ WBC ☐ RBC ☐ INVOICES	☐ DIFF ☐ RETIC ☐ PLATELET ☐ SICKLE CELL ☐ SED RATE ☐ OTHER	CHARGE
AGE ☐ MALE ☐ FEMALE			PHYSICIAN ORDERING TEST
LOCATION	REQUESTED BY INITIALS | TIME AM ☐ PM ☐	DATE	TO BE DONE
			TIME-DATE COLLECTED-INITIALS
			REPORT DATE & TECH.

	TEST	(01) WBC X 10^3	(02) RBC X 10^6	(03) HGB GM %	(04) HCT %	(05) MCV M^3	(10) MCH MMG	(11) MCHC %	(81) ANISO	(82) POIK	(83) HYPOCHR	(84) MACRO	(85) MICRO	(86) POLYCH	COMMENTS-OTHER TEST RESULTS	(27) ESR MM	(28) ESR CORR MM
		IF 90 9 RE-DILUTE							(12) BASO	(13) EOS	(14) MYELO	(15) META	(16) BAND	(17) SEG		(21) PLAT X 10^3	(21) RETIC %
									(18) LYMPH	(19) MONO	(20) PLATELET ESTIMATE	(27) NRBC/100 WBC					(26) SICKLE CELL PREPARATION

Directions: Fill out the printout using the following information. On 4-14 of this year, Dr. Richard Romanos ordered a CBC and sed rate on patient Harry Pratt, age 45 years, patient number 3660892, medical record number 561189. D. D. Morris collected the blood at 2:30 P.M. Melba Wood was the medical technologist who performed the tests on the blood with these results: 9,500 WBC; 4,990,000 RBC; 15.6 Hgb; 46.2 Hct. The indices were MCH—31.3, MCHC—33.8, and MCV—92.6 Platelet count was 162,000 with an MPV of 92.6 The differential showed RBC with slight poikilocytosis; a few ovalocytes; adequate platelets; and 89 segmented neutrophils, 2 eosinophils, 5 lymphocytes, and 4 monocytes. The sedimentation rate was 5 mm/hr. Priority is Today.

Name:	Loc:	Room:
Patient Number:	Age:	Sex:
Med. Rec. No.:	Date:	
Doctor:	Time:	
Diagnosis:		

Hematology

Date:
Time:
Priority:

CBC	**Normals** Low High	Units
WBC	4.5 - 11.0	thous
RBC	4.6 - 6.20	mil
Hgb	14.0 - 18.0	g/dl
Hct	40 - 54	%
MCV	82 - 92	fl
MCH	27 - 31	pg
MCHC	32 - 40	%
Platelets	150 - 450	thous
MPV	7.4 - 10.4	fl

Diff—Manual	**Normals** Low High	Units
Seg Neut	50 -65	%
Eosino	0 - 3	%
Lympho	30 - 40	%
Mono	2 - 10	%
Poikilocytosis		
Ovalocytes		
Platelets		

Sed Rate

Date:
Time:
Priority:

	Normals Low High	Units
Sed Rate	0 - 9	mm/hr

Objective: To recognize terms related to serology

Directions: Give the meaning of the following abbreviations:

1. AIDS
2. ARC
3. ELISA
4. FUO
5. HCG
6. HIV

Directions: Locate, list, and define three terms relating to pregnancy.

7.
8.
9.

Directions: The presence of specific antibodies may be detected by tests that demonstrate certain types of results. Locate, list, and define five terms that refer to these conditions.

10.
11.
12.
13.
14.

23

Directions: Match the following definitions and terms by writing the selected letter in the blank.

____ 15. agglutinin

____ 16. antibody

____ 17. antigen

____ 18. atypical

____ 19. buffy coat

____ 20. febrile

____ 21. fluorescent

____ 22. heterophil antibody

____ 23. inactivation

____ 24. rickettsia

____ 25. serum sickness

____ 26. spirochete

____ 27. titer

a. rendering inert by use of heat

b. irregular, not conforming to type

c. slender, spiral, motile microorganism

d. luminous when exposed to other rays

e. highest dilution of serum causing agglutination

f. minute rod-shaped microorganisms between bacteria and viruses

g. condition which develops following administration of antisera or drug therapy

h. specific antigen used in agglutination tests

i. a test for infectious mononucleosis

j. proteins developed within the body in response to a foreign substance

k. light-colored layer of blood between plasma and red blood cells

l. substance that induces the formation of antibodies

m. pertaining to a fever

Objective: To identify and perform tests for syphilis, pregnancy, and other conditions

Directions: Answer the following questions:

1. What is serology?
2. Differentiate between *in vitro* and *in vivo* testing.
3. Antibodies may be detected by serological tests. List seven types of testing.
4. What bacterium causes syphilis?
5. What is the difference between treponemal antibodies and nontreponemal antibodies?
6. Name the three most commonly used tests for syphilis.
7. Which of these tests are nonspecific?
8. Which of the nonspecific tests is most widely used because it is done more easily and rapidly?
9. What is C-reactive protein?
10. When C-reactive protein is present, (a) how soon after onset of the condition did it appear? (b) When does it subside?
11. Name three conditions in which CRP testing might be positive.
12. How may the rheumatoid factor (RF) in the serum of patients with rheumatoid arthritis be detected?
13. Is the rheumatoid factor test a specific test? Explain your answer.
14. List four characteristics of patients with infectious mononucleosis.
15. What are heterophil antibodies?
16. What is the purpose of febrile agglutination tests?
17. List seven febrile diseases commonly tested for by agglutination.
18. What does the ASO test indicate?

19. Explain the flushed skin color in scarlet fever.

20. What test is used to detect lupus erythematosus?

21. What are cold agglutinins?

22. In which disease is the presence of cold agglutinins useful for diagnosis?

23. a. What hormone is measured in pregnancy testing?

    b. How soon does the hormone appear during pregnancy?

    c. What conditions result in false negative tests?

    d. Name four conditions that could require quantitative testing.

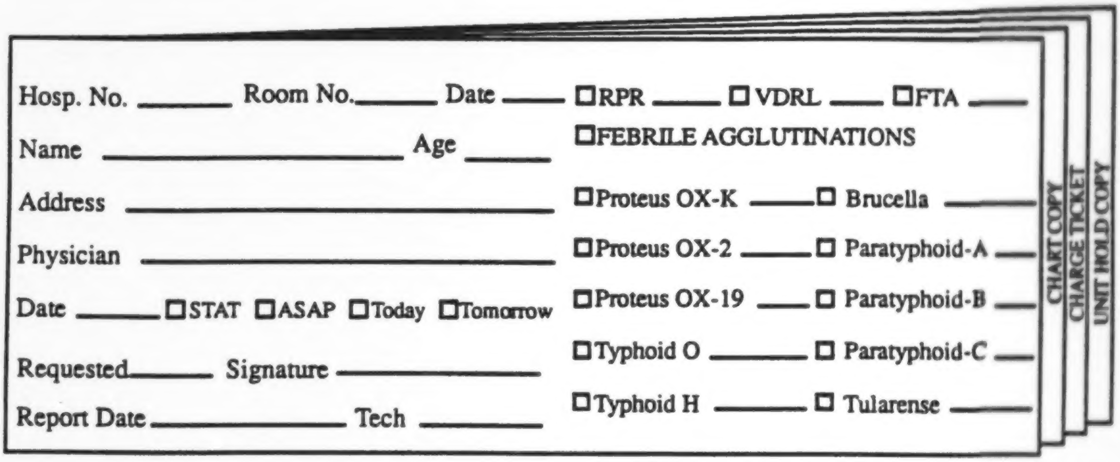
24. Name two tests used to diagnose AIDS.

Learning Activity 3
Completion of a Requisition Form

UNIT 7
Serology

Objective: To complete a form for tests in serology

Directions: Complete the form below for serology using the following information: The patient is Lula Stewart, hospital number 879302, room 202, 23 years old. She lives at 2808 Rio Grande. The test is needed tomorrow. The doctor is Sally Knauer. The following examinations are requested: RPR, febrile agglutinations: Proteus OX-19, Typhoid O, Typhoid H, Brucella, Tularense. Ward Clerk SB signed the request on 4-9-92.



Hosp. No. ______ Room No.______ Date ______ ☐RPR ______ ☐ VDRL ______ ☐FTA ______

Name ______________________ Age ______ ☐FEBRILE AGGLUTINATIONS

Address ______________________ ☐Proteus OX-K ______ ☐ Brucella ______

Physician ______________________ ☐Proteus OX-2 ______ ☐ Paratyphoid-A ______

Date ______ ☐STAT ☐ASAP ☐Today ☐Tomorrow ☐Proteus OX-19 ______ ☐ Paratyphoid-B ______

Requested______ Signature ______________ ☐Typhoid O ______ ☐ Paratyphoid-C ______

Report Date______________ Tech ______ ☐Typhoid H ______ ☐ Tularense ______

CHART COPY
CHARGE TICKET
UNIT HOLD COPY

Learning Activity 1
Terms Relating to Microbiology

Objective: To recognize and use terms relating to microbiology

Directions: Write the correct words in the following blanks.

1. Bacteria are grown on a nutrient substance called a 1. ______________
   a. The liquid nutrient substance is a. ______________
   b. The solid substance is b. ______________
2. A parasitic fungus growing on the skin is a 2. ______________
3. The vegetative structure of the fungus is the 3. ______________
4. A single strand of the vegetative structure is a 4. ______________
5. The study of fungi is called 5. ______________
6. If a fungus produces a disease, it is called 6. ______________
7. If a fungus exists in a mutually beneficial state with another organism, this relationship is called 7. ______________
8. The study of bacteria is 8. ______________
9. Some bacteria require oxygen to grow. These bacteria are 9. ______________
10. Other bacteria grow with no oxygen present. These are 10. ______________
11. Bacteria are grown on artificial media. This growth of bacteria is called a 11. ______________
12. Cultures are usually grown in a shallow, round dish called a 12. ______________
13. Once a culture grows, it can be tested to determine which antibiotics prevent its growth. This process is called 13. ______________
14. The incubation temperature to give the most rapid growth is the 14. ______________
15. Organisms normally present in a body area are the 15. ______________

16. A wire loop is used to transfer bacteria to a slide. The loop is called the 16. ______________

17. Fastening bacteria to a slide to prevent their washing off is called 17. ______________

18. Foreign, unwanted organisms growing in a culture are 18. ______________

19. The process used to keep a surface free of all bacteria is called 19. ______________

Learning Activity 2
Types of Microorganisms and Their Culture

Objective: To collect, inoculate, streak, and store cultures

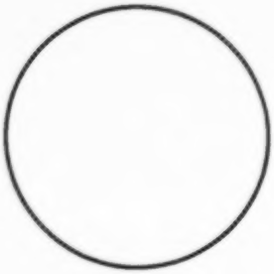
Directions: Write the answers to the following questions.

1. Define *microbiology*.
2. List five types of microorganisms studied in microbiology.
3. What is the responsibility of the microbiology department?
4. Usually, if a bacteriology specimen cannot be processed within an hour, it should be refrigerated. Why?
5. Why is spinal fluid (CSF) for bacteriological study not refrigerated?
6. Name two types of urine specimens suitable for bacteriological study.
7. Describe the special care given to specimens collected on swabs.
8. Why must culture media be sterile before inoculation with a specimen?
9. Name six nutritional requirements of bacteria.
10. Name six types of media.
11. What should be included on a specimen label?
12. Why are cultures in petri plates stored upside down in the incubator?
13. Name four categories of bacteria according to their oxygen requirements.
14. Name three different kinds of equipment that may be used to obtain a reduced oxygen atmosphere.
15. List the three basic shapes of bacteria.
16. Name four types of round bacteria.
17. List and describe three types of curved or spiral forms of bacteria.

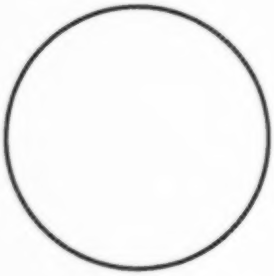
Learning Activity 3
Streaking and Transferring Specimens

Objective: To use the proper technique to transfer specimens to culture media

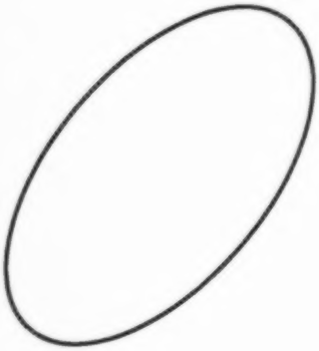
1. Describe the process for streaking a culture plate with a swab. Draw the placement of the streaks within the circle, which represents a dish of culture medium.



2. Describe the process for streaking a culture plate with a urine specimen or other liquid. Draw the placement of the streaks within the circle, representing a dish of culture medium.



3. Describe the process for streaking an agar slant. Draw the placement of streaks within the oval, representing the surface of an agar slant.



Objective: To apply susceptibility discs

Directions: Write answers to the following questions:

1. What is meant by susceptibility testing?
2. List two reasons why it is necessary to press antibiotics discs onto the culture.
3. Look at the report form for susceptibility testing shown in figure 6.
   a. Select and list five broad-spectrum antibiotics used for testing.
   b. Select and list three antibiotics used for testing against Gram-negative organisms.
   c. Select and list two antibiotics used for testing against Gram-positive organisms.
4. Explain what a broad-spectrum antibiotic is. Consult a *Taber's Cyclopedic Medical Dictionary* or talk with the supervisor at the facility where you are assigned.
5. List other broad-spectrum antibiotics used at the facility where you are assigned not included on the form in figure 6.
6. How are findings from this testing reported?

Objective: To prepare and stain slides

Directions: Give complete answers to the following questions.

1. Look at figure 7. Determine what should precede the first step shown in the figure.
2. a. What color are Gram-positive organisms?
   b. Gram-negative?
3. List reagents needed to perform a Gram's stain.
4. What is the purpose of iodine in the Gram-staining procedure?
5. What substances do acid-fast bacteria contain that are not present in most bacteria?
6. What is unusual about the procedure for acid-fast stains?
7. What is the purpose of the 10% KOH in a fungus slide preparation?
8. Why are fungus studies performed under the hood?
9. List seven types of fungus infections.
10. What stain is used on fungi?
11. When should you flame the inoculating loop?

Objective: To use terms relating to chemistry

Directions: Use "Terms and Definitions" to supply answers to the questions.

1. What is automation?

2. Select, list, and define four terms that name pieces of equipment.

3. Explain what the following abbreviations mean:

    a. O.D.

    b. POL

4. The portion of a beam of light absorbed by a substance is measured as O.D. Explain what is meant by the *percentage of transmittance* of this same substance.

5. Both O.D. and percentage of transmittance must be compared to a standard solution. Explain why.

6. What is the purpose of an enzyme?

7. The following terms relate to quality control. Explain what each means.

    a. mean

    b. range

    c. reproducibility

    d. shift

    e. trends

    f. variability

Objective: To describe equipment and procedures used in manual and automated methods

Directions: Write the answers to the following questions.

1. Explain the purpose of the chemistry department.
2. Name three pieces of equipment generally used to measure volume in the laboratory. Refer to figure 1.
3. Why should one always add acid to water?
4. How should you store reagents that are prepared when purchased?
5. Name two methods used to purify water.
6. List the five general components (parts) of an automated laboratory system.
7. Even in automated laboratory systems, the human element continues to be present. Explain.
8. List four factors that might determine whether a laboratory uses automated procedures or not.
9. Name two types of wavelength selectors.
10. Name the five major parts of a photometer.
11. What are two important things for the medical laboratory student to remember in using the photometer?
12. What is *point of care* testing?
13. Name the two major systems of automated clinical laboratory equipment.
14. Which type employs air bubbles or reagents to separate samples?
15. Which type employs centrifugal force to mix samples and reagents?
16. Which type employs dry reagents on a slide or strip?
17. Give another name for the system using dry, layered reagents to react with the sample.

Objective: To describe the purposes of chemical tests

Directions: Answer the following questions, using the last portion of the unit.

1. Of what value is quality control in the laboratory?
2. List five things that must be checked to ensure quality control standards.
3. Differentiate between trends and shifts.
4. How is glucose used in the body?
5. What is a postprandial test?
6. Name five tests that may give an indication of kidney disease.
7. Name five tests that may give an indication of liver disease.
8. Name four different lipids.
9. How are calcium and phosphorus used in the body?
10. List the four tests that make up the electrolyte series.
11. Explain the difference in the HDL and LDL portions of cholesterol.
12. What is the range for blood pH?
13. Name eight enzymes. Tell where each is found, or what disease condition it relates to.
14. What is a profile?
15. What is electrophoresis?
16. Why do charged particles move at different velocities?
17. Name two body products which can be assayed using electrophoresis.
18. Determine reasons for drug monitoring.

Learning Activity 4
Quality Control—Variability

UNIT 9
Chemistry

Objective: To study variability in test results

Directions: Obtain three cards of different sizes (3x5, 4x6, 5x8, etc.). Using a metric ruler, measure each card to the nearest 0.1 mm. Then have at least four other students measure the same cards. Record all results, and cover your answers as they are recorded. After all students have completed the assignment, check the results to determine (1) the range and (2) the mean.

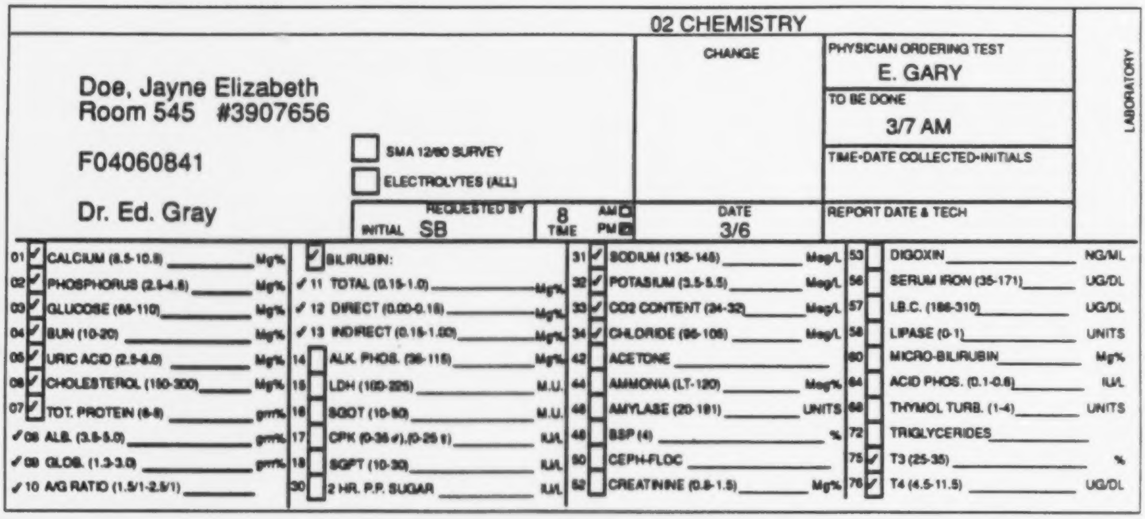
STUDENT	*Card 1*	*Card 2*	*Card 3*
1.			
2.			
3.			
4.			
5.			
6.			

Range:

Mean:

Objective: To report results of chemistry tests

Directions: The form below was received in the laboratory. The specimen was collected by D. M. at 7:15 A.M. on 3-7-(this year). After all tests were completed, B. Y. wrote the results on the form to be returned to the patient's chart. The charge was $50.00. These were the results: glucose = 105 Mg%; cholesterol = 175 Mg%; total protein = 7 gm% with albumin = 4.8 gm%; globulin = 2.2 gm% and A/G ratio = 2.18/l; phosphorus = 4 Mg%; calcium = 10.2 Mg%; bilirubin = 0.5 Mg% with 0.05 Mg% direct and 0.45 Mg% indirect; blood urea nitrogen (BUN) = 12 Mg%; uric acid = 2.8 Mg%; T_3 = 26%; T_4 = 8.4µG/Dl; chloride = 98 Meg/L; CO_2 = 27 Meg/L; sodium = 138 Meg/L; potassium = 3.9 Meg/L. Fill out the form as it would appear when B. Y. finished.



02 CHEMISTRY

Doe, Jayne Elizabeth
Room 545 #3907656

F04060841

Dr. Ed. Gray

☐ SMA 12/60 SURVEY
☐ ELECTROLYTES (ALL)

REQUESTED BY INITIAL SB

TIME 8 AM ☐ PM ☒

CHANGE

DATE 3/6

PHYSICIAN ORDERING TEST E. GARY

TO BE DONE 3/7 AM

TIME-DATE COLLECTED-INITIALS

REPORT DATE & TECH

LABORATORY

01 ☑ CALCIUM (8.5-10.5) ________ Mg%
02 ☑ PHOSPHORUS (2.5-4.5) ________ Mg%
03 ☑ GLUCOSE (65-110) ________ Mg%
04 ☑ BUN (10-20) ________ Mg%
05 ☑ URIC ACID (2.5-8.0) ________ Mg%
06 ☑ CHOLESTEROL (150-300) ________ Mg%
07 ☑ TOT. PROTEIN (6-8) ________ gm%
✓ 08 ALB. (3.5-5.0) ________ gm%
✓ 09 GLOB. (1.3-3.0) ________ gm%
✓ 10 A/G RATIO (1.5/1-2.5/1) ________

☑ BILIRUBIN:
✓ 11 TOTAL (0.15-1.0) ________ Mg%
✓ 12 DIRECT (0.00-0.15) ________ Mg%
✓ 13 INDIRECT (0.15-1.00) ________ Mg%
14 ☐ ALK. PHOS. (36-115) ________ Mg%
15 ☐ LDH (100-225) ________ M.U.
16 ☐ SGOT (10-50) ________ M.U.
17 ☐ CPK (0-35 ♂),(0-25 ♀) ________ IU/L
18 ☐ SGPT (10-30) ________ IU/L
30 ☐ 2 HR. P.P. SUGAR ________ IU/L

31 ☑ SODIUM (135-145) ________ Meg/L
32 ☑ POTASIUM (3.5-5.5) ________ Meg/L
33 ☑ CO2 CONTENT (24-32) ________ Meg/L
34 ☑ CHLORIDE (95-105) ________ Meg/L
42 ☐ ACETONE ________
44 ☐ AMMONIA (LT-120) ________ Meg%
46 ☐ AMYLASE (20-181) ________ UNITS
48 ☐ BSP (4) ________ %
50 ☐ CEPH-FLOC ________
52 ☐ CREATININE (0.5-1.5) ________ Mg%

53 ☐ DIGOXIN ________ NG/ML
56 ☐ SERUM IRON (35-171) ________ UG/DL
57 ☐ I.B.C. (186-310) ________ UG/DL
58 ☐ LIPASE (0-1) ________ UNITS
60 ☐ MICRO-BILIRUBIN ________ Mg%
64 ☐ ACID PHOS. (0.1-0.8) ________ IU/L
66 ☐ THYMOL TURB. (1-4) ________ UNITS
72 ☐ TRIGLYCERIDES ________
75 ☑ T3 (25-35) ________ %
76 ☑ T4 (4.5-11.5) ________ UG/DL

ST COPY AVAILABL

Learning Activity 1
Terms Relating to the Blood Bank

Objective: To use terms associated with the blood bank

Directions: Match the term to the definition by writing the letter in the blank.

_____ 1. antigens found on the surface of red blood cells

_____ 2. precipitate formed when plasma is frozen

_____ 3. liquid portion of blood

_____ 4. the entire unit of blood

_____ 5. antibodies in plasma which react with agglutinogens

_____ 6. someone who gives blood

_____ 7. concentrated solution of blood cells used for transfusion

_____ 8. donation of blood by a person for his/her own transfusion

_____ 9. compound of iodine with a stabilizing agent

_____ 10. person who receives blood or a blood component

_____ 11. portions of blood separated from a single unit

_____ 12. replacement of whole blood or its components

_____ 13. tests performed to determine whether a donor's blood will be compatible with the recipient's blood during transfusion

a. agglutinins

b. agglutinogens

c. autologous blood

d. components

e. cross match

f. cryoprecipitate

g. donor

h. iodophore

i. plasma

j. recipient

k. red blood cells

l. transfusion

m. whole blood

Objective: To outline the procedure for drawing, processing, and labeling donor blood

Directions: Answer the following questions:

1. Why are transfusions necessary?
2. What age restrictions are placed on blood donors?
3. Explain the difference in donor rejection and being deferred. Give several examples of each.
4. State other requirements which must be met before a donor's blood can be drawn.
5. Describe the process of preparing a person's arm for a blood donation.
6. What anticoagulant is usually found in the collecting unit?
7. How much blood will be place in a collection unit?
8. What is a pilot tube? For what is it used?
9. Besides a pilot tube, how are other blood samples made available for testing and cross matching donor blood?
10. List places and records where a donor number will appear.
11. At what temperature is blood stored?
12. For what is each unit of donor blood tested to assure quality?
13. List items which must appear on the label on a unit of blood.
14. How often is stored blood inspected?
15. If you planned elective surgery in the near future, could you provide your own blood for a transfusion, if one were needed? What type of blood is this called?

Objective: To determine ABO blood group and Rh factor and explain how blood is cross matched

Directions: Answer the following questions from the last portion of the unit:

1. Explain the relationship between antigens and antibodies.
2. What are antigens and antibodies in the blood called, and where are they located?
3. What determines blood type?
4. How many agglutinogens are used in typing blood groups? Name these.
5. What type of agglutinins will be found in the serum of someone with type A blood?
6. At what age is the blood type established, and when does it change?
7. Is the Rh factor an antigen or antibody, and where is it located?
8. Name two occasions when the Rh factor is important.
9. Explain why the Rh factor becomes important in transfusions.
10. Describe conditions which occur causing hemolytic disease of the newborn (erythroblastosis fetalis).
11. What can be done to prevent this condition?
12. List seven components which may be derived from a unit of whole blood.
13. List three tests performed to determine compatibility during the cross-matching process.
14. Explain the purpose of the Coombs test.
15. What information must be recorded in the transfusion release ledger?

Objective: To ascertain the number and type of units present in the blood bank

Directions: Ask your supervisor to allow you to observe as the blood supply is inspected and inventoried. If this is not possible, ask permission to count and record the number and types of units present. Remember that this can be done with the door closed to ensure that the bank's temperature is not raised. Record the totals on the form below.

TYPE	*Positive*	*Negative*
A		
B		
AB		
O		

Directions: Give the name and location of this blood bank.

Give the names and locations of other blood banks in the same city or in the same area.

Learning Activity 1
Terms Relating to Histology/Cytology

UNIT 11
Histology/Cytology

Objective: To use terms relating to both cytology and histology

Directions: Match the following terms to the definitions, and write the letter in the blank.

____ 1. cells that have been shed from the body

____ 2. living tissue removed for microscopic diagnosis

____ 3. identification of tissue by its color reaction to dyes

____ 4. solution to prevent decomposition of tissue

____ 5. autopsy

____ 6. Pap smear

____ 7. study of cells

____ 8. study of tissue

____ 9. solution to preserve tissue

____ 10. examination of a dead body

____ 11. place tissue sections on slides

____ 12. freezing tissue for immediate sectioning, staining, and diagnosis

____ 13. machine with a knife for cutting tissue in thin slices

____ 14. removal of water

a. autopsy

b. biopsy

c. cytology

d. dehydration

e. exfoliated cells

f. frozen section

g. fixative

h. histology

i. microtome

j. mount

k. Papanicolaou smear

l. postmortem

m. staining

Objective: To receive, process, and store slides of tissues and cells with acceptable techniques and safety precautions

Directions: Answer the following questions.

1. List three occasions when tissues are obtained for examination.

2. Specimens may arrive in a form other than a tissue. List two additional types of specimens received in histology.

3. Describe what occurs during a gross examination.

4. List the main steps in the process of placing tissue on slides for study.

5. State the ideal size of a tissue sample for processing.

6. What is infiltration of tissue?

7. How is sectioning accomplished?

8. Explain why the microtome knife blade must be sharp.

9. What is a "ribbon," and how is it formed?

10. Cite two dyes most commonly used, and explain how they stain cells.

11. What is a counterstain?

12. Would a worker in histology be expected to provide the same stains as are available in hematology and microbiology, if needed?

13. List four special stains used in the pathology department.

14. Define the following terms:

    a. clearing

    b. mounting

15. What is the purpose of the Papanicolaou smear?

16. Discuss different methods and purposes for slide storage.

17. What problems must be considered in labeling specimens in histology/cytology?

18. What could happen to small bits of tissue which are not wrapped together?

19. What precautions does the CDC require of persons performing postmortem procedures?

20. List three stains or techniques used in the facility where you are assigned that were not mentioned in this unit.